Target and suspect screening of organic micropollutants and their transformation products in aqueous samples

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> Nothing is impossible, the world itself says ‘I’m possible’!

Audrey Hepburn

Abstract

The presence of organic micropollutants in wastewater imposes a problem to the water treatment industry. Such persistent compounds enter the sewer system after domestic or medical use, and are inefficiently removed by traditional wastewater treatment technologies and subsequently discharged into the aquatic environment.

Chemical oxidation using ozone has been proven as an effective treatment process for a wide spectrum of micropollutants during bench-, pilot- and full-scale experiments in both wastewater and drinking water. However, a major disadvantage of ozonation is the formation of transformation products (TPs) instead of a full mineralization of parent compounds. Although there is still an overall lack of information regarding their toxicity, bioaccumulation, or occurrence, many of these compounds are suspected to have potential effects on humans and other species.

A proper sample preparation method is required to enrich a wide range of micropollutants from water samples for a subsequent use in chemical analysis as well as toxicological evaluation. Solid phase extraction (SPE) has become the most common sample preparation technique in environmental analysis.

In the first stage of research, the performance of several commercial SPE materials belonging to three different groups: reversed-phase, mixed-mode anion exchanger and mixed-mode cation exchanger, was evaluated. Eight parent compounds and seventeen ozonated TPs with different physicochemical properties were extracted from pure water samples. Different pH values, washing and elution solvents were tested to optimize the procedure. Recoveries ≥ 91% were obtained by combining mixed-mode strong anion and cation exchangers in tandem without pH adjustment.

A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed to analyze the compounds of interest using the optimized SPE procedure. Full validation followed by trace determination of target compounds in different water matrices was performed. Consequently, recoveries between 90 and 110%, Linearity (> 0.99), method quantification limits (MQL's) at low ng/L-range and low matrix effect (ME) were achieved.

In the second stage of the study, suspect screening approach was used to examine the presence of structurally diverse organic compounds and their ozonated TPs in environmental water samples without reference standards. The suspect list was assembled after an extensive search to include 245 candidates reported form laboratory experiments in literature. The analytical procedure was therefore optimized by combining liquid chromatography-quadrupole-time-of-flight-mass spectrometry (LC-Q-TOF-MS) based on the use of accurate mass with the optimized SPE method

exhibiting broad enrichment efficiency. The relative concentration levels of the suspects were determined and compared according to their peak areas in several advanced wastewater samples at different treatment levels and the final receiving water. Mass accuracy < 5 ppm, isotopic score ≥ 80% and peak height > 1000 counts were obtained for all detected suspects. In addition, a plausible matching was shown between the retention times of TPs relative to parent compounds and the available values from literatures. The results showed that the studied wastewater treatment plant was efficient to degrade partially or completely organic micropollutants and the formed TPs after advanced treatment.

The future focus will be to study the occurrence and toxicological relevance of various compound classes and TPs in different aquatic environments using the improved SPE procedure. Additionally, further confirmation of suspect screening data will be beneficial.

Kurzfassung

Das Vorkommen organischer Spurenschadstoffe im Abwasser stellt eine große Herausforderung für die Abwasseraufbereitungsindustrie dar. Die Schadstoffe gelangen nach häuslichem Gebrauch von Hygieneprodukten und Medikamenten in die städtischen Abwassersysteme und zu den kommunalen Kläranlagen. Da eine Vielzahl dieser Schadstoffe nicht durch traditionelle Abwasseraufbereitung abgebaut oder entfernt werden können, gelangen die Schadstoffe nach dem Passieren der Kläranlagen in die Umwelt.

Eine effektive Methode für den Abbau dieser Spurenschadstoffe ist die chemische Oxidation durch Ozon. Untersuchungen von Laboranlagen bis hin zu Pilotanlagen haben die Effizienz des Schadstoffabbaus durch Verwendung von Ozon in der Abwasserbehandlung und der Trinkwasseraufbereitung gezeigt. Dennoch bringt die Ozonbehandlung wesentliche Nachteile mit sich. Eine Vielzahl der Schadstoffe wird nicht vollständig mineralisiert, sondern nur teilweise abgebaut. Diese sogenannten Transformationsprodukte (TP) stellen ein bis heute ungeklärtes Risiko für den Menschen und die Umwelt dar. Nur wenig ist bekannt über das Vorkommen und Entstehen, die Toxizität und die Bioakkumulation dieser TPs.

In Anbetracht der geringen Konzentrationen der Spurenschadstoffe, erfordert die Analyse der Umwelteinflüsse eine effiziente Anreicherungsmethode, die in der Lage ist, ein großes Spektrum an TPs aus verschiedenen Matrizes zu binden. Hierbei stellt die Festphasenextraktion eine der am häufigsten verwendeten Extraktionstechniken im Bereich der Umweltanalyse dar.

Im ersten Schritt dieser Forschungsarbeit wurden verschiedene handelsübliche SPE Materialien aus drei verschiedenen Klassen getestet: reversed-phase, mixed-mode anion exchanger und mixed-mode cation exchanger. Acht Hauptverbindungen mit verschiedenen physikochemischen Eigenschaften und 17 nach Ozonbehandlung entstandene TPs wurden für die Evaluation der Anreicherungseffizienz verwendet. Im weiteren Verlauf der Untersuchung wurden die pH-Werte und die Wasch- und Elutionsmittel variiert um eine optimierte Anreicherung zu erhalten. Wiederfindungsraten von ≥ 91% wurde unter Verwendung einer Kombination aus im Tandem verwendeten mixed-mode strong anion und cation exchanger Materialien ohne pH-Wert Einstellung erreicht.

Die Analyse der mit Hilfe der optimierten SPE Methode angereicherten Hauptkomponenten und TPs erfolgte über eine eigens entwickelte Flüssig Chromatographie Tandem Massen Spektrometer (LC-MS/MS) Methode. Die Validierung der Methode erfolgte über die Bestimmung der Hauptkomponenten und TPs aus realen Wasserproben mit verschiedenen Matrizes.

Wiederfindungsraten lagen bei 90 bis 110%, die Linearität lag bei R^2 > 0.99 und die Nachweisgrenzen der Methoden lagen im Subnanogramm-Bereich pro Liter. Matrixeffekte konnte in keiner der extrahiert und analysierten Realproben beobachtet werden.

Im zweiten Teil der Studien wurde ein sogenanntes suspect screening durchgeführt. Hierbei wurden die Wasserproben auf das Vorkommen einer Vielzahl von literaturbekannten organischen Substanzen und der korrespondierenden Nebenprodukte nach Ozonierung analysiert. Das suspect screening führte zu 245 potenzielle Kandidaten, welche bereits aus Laborversuchen und wissenschaftlichen Arbeiten bekannt sind. Die Auswahl der potenziellen Kandidaten erfolgte ohne Verwendung von Referenzstandards, daher wurde entschieden, im weiterem Verlauf der Studie die Analysemethode für die Verifizierung der Ergebnisse zu optimieren. Die Optimierung basierte auf der Verwendung eines LC-Q-TOF-MS Systems und basierte auf der Bestimmung der exakten Massen der mit optimierter SPE angereicherten Analyten. Das verwendete LC-Q-TOF-MS System ermöglichte neben dem Nachweis einer hohen Effizienz der Anreicherungsmethode auch die quantitative Analyse zahlreicher Abbauprodukte. Die relativen Konzentrationen der Ursprungssubstanzen und deren potenziellen Nebenprodukte zu verschiedenen Zeitpunkten der Abwasseraufbereitung konnte aus den detektierten Peakflächen abgeleitet werden. Die Massengenauigkeit der verifizierten Suspects lag bei < 5ppm mit einem Isotopenscore ≥ 80% und Peakhöhen > 1000 counts. Als zusätzliche Absicherung wurden die Ergebnisse einer Plausibilitätsprüfung unterzogen, bei der die experimentell erhaltenen Retentionszeiten mit literaturbekannten Werten verglichen wurden.

Die Ergebnisse dieser Arbeit haben gezeigt, dass die untersuchte Kläranlage in der Lage war, mit Hilfe der fortschrittlichen Behandlung durch Ozon eine Vielzahl der organischen Spurenschadstoffe vollständig oder partiell abzubauen.

Zukünftig Studien werden mithilfe der entwickelten SPE Methode das Vorkommen, die Akkumulation und die Ökotoxizität diverser Substanzklassen und derer Transformationsprodukte in der aquatischen Umwelt verifizieren können. Die weitere Identifikation und Bestätigung von suspect screening Daten durch Verwendung der SPE Methode wird hierbei hilfreich sein.

Table of contents

List of abbreviations

AAS	Atomic absorption spectrometry
ACF	Acesulfame
AChE	Acetylcholinesterase
ACN	Acetonitrile
ACV	Acyclovir
AhR	Aryl hydrocarbon receptor
AMP	Aminopyrine
AOPs	Advanced oxidation processes
ATL	Atenolol
BPA	Bisphenol A
BZR	Bezafibrate
BZT	1*H*-benzotriazole
BZR	Bezafibrate
°C	Degree Celsius
CAFF	Caffeine
CAFLUX	Chemically activated fluorescent gene expression
CALUX	Chemically activated luciferase gene expression
CBZ	Carbamazepine
CE	Collision energy
CFX	Ciprofloxacin
CLP	Chlorophene
CMC	Clarithromycin
CPX	Cephalexin
CXP	Cell exit potential
DAD	Diode array detector
DFC	Diclofenac
DNA	Deoxyribonucleic acid
DP	Declustering potential
DW	Drinking water
DWT	Drinking water treatment
DWTP	Drinking water treatment plant

ECD	Electron capture detector
EDCs	Endocrine disrupting compounds
ELISA	Enzyme-linked lmmunosorbent assay
ER	Estrogen receptor
EROD	Ethoxyresorufin O-deethylase
E-SCREEN	Estrogen screen
ESD	17β-Estradiol
ESI	Electrospray ionization
EST-S	Estrone sulfate
Fig	Figure
FLD	Fluorescence detector
FT	Fourier transform
GC	Gas chromatography
GW	Ground water
h	Hour
H_2O	Water
HLB	Hydrophilic-lipophilic balance
HPLC	High performance liquid chromatography
HR	High resolution
HRMS	High resolution mass spectrometry
IC	Ion chromatography
ICP	Inductively coupled plasma
ICR	Imidacloprid
IMZ	Imazalil
IR	Infrared
KPR	Ketoprofen
L	Liter
LC	Liquid chromatography
LDTD	Laser diode thermal desorption
LIT	Linear ion trap
LLE	Liquid-liquid extraction
Log P_{ow}	Octanol-water partition coefficient
LR	Low resolution
LTQ	Linear trap quadrupole

LVX	Levofloxacin
MBZ	Methylbenzotriazole
MDL	Method detection limit
ME	Matrix effect
MeOH	Methanol
mg	Milligram
µg	Microgram
min	Minute
µL	Microliter
mL	Milliliter
MLD	Methylindole
µm	Micrometer
mm	Millimeter
MPL	Metoprolol
MQL	Method quantification limit
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
NA	Not available
NFX	Norfloxacin
n	Number of measurements
ng	Nanogram
ND	Not detected
NMR	Nuclear magnetic resonance
No.	Number
PCM	Paracetamol
PCPs	Personal care products
PG	Penicillin G
PGT	Progesterone
pKa	Acid dissociation constant
PPCPs	Pharmaceuticals and personal care products
PRL	Propranolol
Q	Quadrupole
QqQ	Triple quadruple

R^2	Correlation coefficient
RE	Recovery
ROX	Roxithromycin
RRT	Relative retention time
RSD	Relative standard deviation
RT	Retention time
S/*N*	Signal-to-noise ratio
SCGE	Single cell gel electrophoresis
SMZ	Sulfamethoxazole
SPE	Solid phase extraction
SW	Surface water
TCS	Triclosan
TLC	Thin layer chromatography
TMD	Tramadol
TMP	Trimethoprim
TOF	Time-of-flight
TP	Transformation product
TW	Tap water
UPLC	Ultra-performance liquid chromatography
UV	Ultraviolet
V	Volt
VFX	Venlafaxine
WW	Wastewater
$WWAO_3$	Wastewater after ozonation
$WWBO_3$	Wastewater before ozonation
WWFE	Wastewater final effluent
WWTP	Wastewater treatment plant
YES	Yeast estrogen screen

List of figures

List of tables

1 General introduction

1.1 Background

The growing scarcity of water resources is one of the most critical environmental problems facing us in the near future. A long-lasting sustainability of safe water supply is regulated by stringent protection and management of water resources and an efficient reclamation of used water from different effluents. Recently, the pollution of water compartments by organic micropollutants such as pharmaceuticals and personal care products (PPCPs), flame retardants, pesticides, and endocrine disrupting compounds (EDCs) has attracted increased attention from scientific research and consequently public awareness. Many of these compounds persist at least partially during conventional wastewater treatment (WWT) and were detected in secondary effluents and receiving surface waters (SWs) worldwide [1-5]. Thus, residues of these compounds might reach drinking water (DW) and cause potential risk on human health due to their biologically active nature if drinking water treatment (DWT) is not able to remove them completely.

The presence of low concentrations of PPCPs has been associated with endocrine disruption [6], chronic toxicity [7, 8], and even the development of pathogen resistance [9]. At present, there are no legal regulations established to ensure these substances or new compounds and by-products from being discharged into SW bodies [10-13].

Studies conducted in various parts of the world show the presence of micropollutants in potable water sources [14-18]. The presence of drugs in the German aquatic environment at concentrations up to 1 µg/L was reported [19]. Clofibric acid has been found in DW at concentrations of up to 165 ng/L [20]. From 1996 and 1998, a comprehensive German study investigated the occurrence of 55 pharmaceuticals, 6 hormones, 9 metabolites, 6 biocides and 1 flame retardant in the discharges from 49 wastewater treatment plants (WWTPs) and in their respective receiving water bodies [21]. Concentrations at the µg/L level of 32 pharmaceuticals, 4 hormones, 5 metabolites, and 5 biocides were detected in the WWTP outflow. The receiving water bodies contained concentrations of beta-blockers and anti-epileptic agents in excess of 1 µg/L. Clofibric acid, diclofenac, ibuprofen, propyphenazone, primidone and carbamazepine were detected in the influent and effluent of municipal WWTPs at concentrations up to the µg/L level, as well as in groundwater (GW) aquifers near sources of contaminated water [22]. Estrone, 17β-estradiol, and 17α-ethinylestradiol were studied in the water cycle of Berlin, Germany [23]. Detection limits in DW, SW, and WW effluent ranged from 0.1-0.4 ng/L. All three compounds were present in

influent WW at concentrations of 8.6-160 ng/L. Only E1 was detected in SWs and in GW, at levels around 1 ng/L and 0.1 ng/L, respectively. Two polycyclic musk fragrances galoxolide (1900 ng/L) and tonalide (580 ng/L) were measured in German influent WWTP [24]. Several compounds that were previously unreported in SWs were detected in the Lippe River, Germany and attributed to anthropogenic inputs [25, 26]. These compounds include a plasticizer (2,2,4-trimethyl-1,3-pentandioldiisobutyrate) at up to 100 ng/L and a surfactant (2,4,7,9-tetramethyl-5-decyne-4,7-diol) at up to 660 ng/L. Numerous other PPCPs were also detected.

Humans and animals treated with pharmaceuticals are the main source of micropollutants in the environment, although the points where these are released are diverse [27]. Hospitals and households are the main locations where PPCPs enter the WWTPs [28, 29]. Other sources of micropollutants in the environment include industrial discharge, septic tanks, which can directly contaminate GW [30], and pharmaceuticals used as growth promoters and feed additives in agriculture and aquaculture [31]. Land application of livestock manure may cause contamination by runoff into SWs and leaching into the GW. Similarly, landfills may be a source of contamination, if they contain disposed drugs or sewage sludge with sorbed chemicals [27], while households, hospitals and other facilities (e.g. schools, residential institutions) are the most significant points of release [32]. The most important routes of contamination of micropollutants into the environment are depicted in Fig. 1.1.

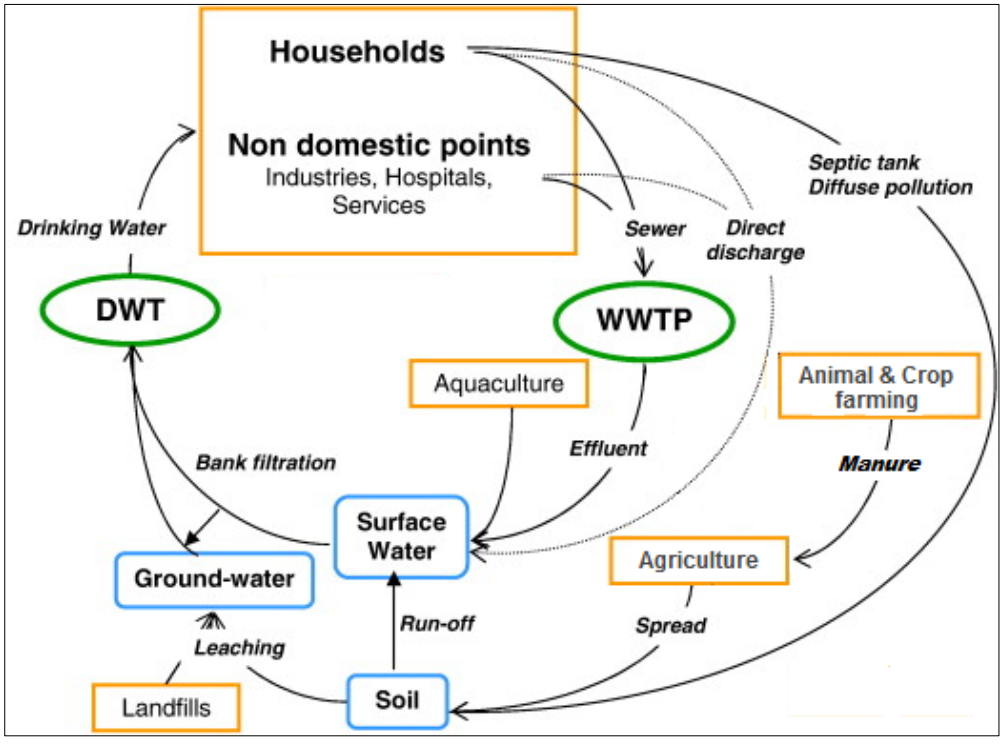

Figure 1.1: Origin and routes of micropollutants [33]

Municipal WWTPs are an important point source of micropollutants released into the environment and water bodies [14, 33]. A study stated that in 264 municipal WWTPs around the world, 118 pharmaceutical compounds belonging to 17 different classes were found in the effluents [13].

The conventional technologies to treat WW are efficient in removing suspended solids and nutrients. However, they are not effective in removing the micropollutants that are present in trace quantities [21]. Hence, new treatment technologies or additional treatment processes are required to remove these compounds.

The use of chemical oxidation procedures can constitute effective technologies for the removal of unwanted substances present in waters. Among these oxidation procedures, single oxidants such as chlorine, UV irradiation, hydrogen peroxide, and ozone, or combinations of these oxidants in the advanced oxidation processes (AOPs), such as UV/H_2O_2, O_3/H_2O_2, UV/TiO_2, and Fenton/photo-Fenton systems are frequently applied [34, 35].

Ozone and ozone based AOPs were shown to be effective in the oxidation of micropollutants both in water and WW matrices [36, 37]. The oxidation process occurs either directly or via the formation of hydroxyl and other radicals [38, 39]. This formation of radicals makes ozone a potent oxidant and effective agent to remove pharmaceuticals and other micropollutants from WW. Several studies have confirmed that ozone treatment can be very efficient in the oxidation of a wide range of micropollutants featuring electron-rich moieties such as activated aromatic rings, amine functions and double bonds (e.g. beta blockers, antibiotics, estrogens, anti-inflammatory drugs, plasticizers, flame retardants) [39-44]. Even for a medium ozone dose of 0.6 g O_3 g^{-1} dissolved organic carbon, high removal rates (> 85%) have been observed for many micropollutants with different functional groups in a municipal WWTP upgraded with a full-scale post-ozonation [41]. In addition, ozonation has been found to reduce or to eliminate the pharmacological and biological effects of micropollutants [45]. Due to the significant advances in the ozone manufacturing technology in the last couple of decades and the experience gained by ozone treatment of water and WW, ozonation is now a mature technology [46]. These developments have led to a huge surge in research related to ozone treatment of secondary and tertiary treated municipal WW the world over in recent years. A study showed that municipal WW effluents spiked with 11 selected PPCPs and treated with ozone in a pilot-scale were oxidized as much as 90–99% at ozone doses ranging from 2 to 5 mg/L [47]. Removals greater than 90% were reported with ozone doses ranging from 0.1 to 30 mg/L for a vast range of compounds (pesticides, anti-inflammatories, antiepileptics, antibiotics and natural and synthetic estrogens) [48]. The impact of ozone was studied in 84 pollutants present in a secondary effluent from a conventional WWTP [49]. The contaminants analyzed included pharmaceuticals (analgesics, antidepressants,

anti-inflammatory, antibiotics, antiepileptics, beta-blockers and lipid regulators among others), PCPs (sunscreen agents, synthetic musks), stimulants (caffeine, nicotine) and some metabolites (clofibric acid, cotinine, several metabolites of dipyrone). The results showed high removals for most of the compounds.

However, despite the high reactivity of ozone, recent studies revealed that ozonation of WW can lead to the formation of transformation products (TPs) with toxicophoric structures such as aldehyde and bialdehyde moieties [50] as well as to considerable developmental retardation of rainbow trouts (*Oncorhynchus mykiss)* [51]. For example, ozonation of water resources containing N,N-dimethylsulfamide, which is a biological TP of the fungicide tolylfluanide, has been recently shown to lead to the formation of carcinogenic N-nitroso-dimethylamine [52].

Since most TPs have a higher environmental mobility and persistence and, they are favored to occur in DW resources [4]. However, for most micropollutants the formation and identity of TPs and therefore their occurrence in water resources and finished DW is currently unknown.

Many laboratory studies have proposed TPs after ozonation of different organic compounds but without screening their possible occurrence and distribution in environmental samples due to the lack of reference standards and a proper sample preparation technique which facilitates their detection as well as toxicological evaluation.

The identification of micropollutants and TPs is highly challenging considering the large number of anthropogenic chemicals emitted intentionally or unintentionally into the environment. The different approaches used for the identification of these compounds in environmental water samples are classified in three principal categories (target analysis, suspect screening, and non-target screening) [53].

For target analysis, a reference standard is necessary to determine the analyte concentration in the sample and to match the measured retention time (RT). A complete target analysis cannot be performed for all compounds of potential environmental relevance, as this would involve the purchase and measurement of hundreds, if not thousands, of chemicals for which reference standards are not always available. Thus, when analyzing complex samples, a balance is needed between extensive target analysis and screening methods, which can assist in tentatively identifying other potentially relevant compounds. Suspect screening relies on accurate mass and isotope information available for the precursor ion and additional evidence for tentative identification. Compounds that are expected to be in the samples (the “suspects”) can be screened using the exact mass of their expected ions, calculated from the molecular formula. Nontarget screening involves masses that are detected in the samples, but where no a priori information on

the underlying compound is available beforehand. Full identification of the nontarget mass is often difficult, with no guarantee of a successful outcome [53, 54]. High accuracy, high resolution data improve the chances of a unique molecular formula assignment to detected masses [55].

Currently, the detection of both micropollutants and TPs requires the use of chromatographic techniques hyphenated to mass spectrometry (MS). Due to the polar nature of TPs, the most commonly used separation technique is liquid chromatography (LC) [56, 57]. Different reviews have presented and discussed the use of LC-MS based techniques for the determination of micropollutants and their TPs in aqueous environmental samples [56, 58-60].

Until recently, LC-MS-MS instruments with triple quadruple (QqQ) analyzers have been the most widely employed for quantitative target compounds analysis. But even though the sensitivity, selectivity, and efficiency characteristics of multiple reaction monitoring (MRM) approach are excellent, qualitative information needed to support the structural elucidation of analytes is lost [61]. In addition, QqQ instruments can only measure nominal masses, and when they are operated in the full scan mode, the sensitivity is low, restraining the analysis to a given number of analytes. High-resolution MS (HRMS) transcends the major limitations of LC-MS/MS systems for both suspect and non-target analysis. HRMS instruments like time-of-flight (TOF) or Orbitrap provide high-quality information by combining full mass spectrum data with high mass resolution and mass accuracy [62, 63]. In theory, the presence of an unlimited number of compounds can be investigated at the proper sensitivity, without requiring the preselection of analytes or even without having reference standards available.

1.2 Sample preparation

To analyze complex mixtures, such as water samples, a pretreatment procedure is useful to provide a sample fraction enriched with all the target analytes and as free as possible from other matrix components [64].

A survey showed that sample preparation accounted for nearly 61% of the time required to conduct an analytical task [65]. The operating principle of any sample preparation method is to allow analytes to partition between sample matrix and an extraction phase.

The basic concept of sample preparation is to convert a real matrix into a sample suitable for analysis. Even the best analytical techniques cannot rectify problems generated by sloppy sample pretreatment. The main goals of sample preparation include removing of potential interferences, increasing the concentration of target analytes, producing a sample aliquot that will not damage

the column or instrument and providing a robust, reproducible method that is independent of variations in the sample matrix [66].

The best established methods to perform an accurate and precise environmental analysis are liquid-liquid extraction (LLE) and solid phase extraction (SPE) techniques.

LLE uses two immiscible solvents to transfer the analytes from one media to the other. Although LLE has been used as a sample preparation procedure for analysis of trace organics for decades, it has become less popular over time.

In addition to emulsion formation, difficulty in automation, and time consumption, LLE also requires large volumes of organic solvents, some of which are toxic and can also be expensive. LLE is a multi-step procedure that often results in loss of analytes during the process, frequently making sample preparation the major source of errors in the analysis, and making it impeditive for integration with the rest of the analytical process [67]. SPE, on the other hand, can overcome all of these drawbacks.

From trace levels to industrial scale, SPE plays an important role in a broad range of applications. SPE refers to the exhaustive removal of chemical constituents from a flowing liquid sample via retention on a contained solid sorbent and subsequent recovery of selected constituents by elution from the sorbent. It is an increasingly being used sample preparation technique.

SPE is the method of choice that is particularly well adapted to multi-residue analysis, including compounds with a wide range of polarity or characterized by various physicochemical properties [68].

Over time, SPE has been developed into different formats. The most common format of SPE is in form of a cartridge. Sorbent particles (nominally 50 μm in diameter) are packed with two polyethylene fritted disks above a male Luer tip in a disposable short column (generally an open polypropylene syringe barrel) that acts as a reservoir for the environmental samples and solvents, as seen in Fig. 1.2.

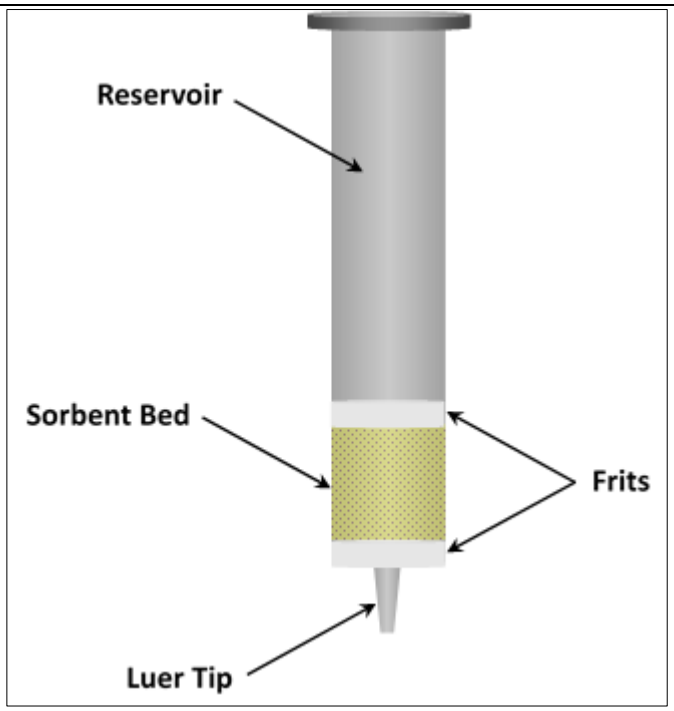

Figure 1.2: Typical SPE cartridge

SPE disks were first designed to treat large sample volumes with a higher flow rate than cartridges and to avoid blockages caused by suspended particles and matrix components [69]. SPE cartridges and disks share the same sorbent technology and the only difference between these two devices is the format. Cartridges can be easily fabricated in a laboratory environment, however, disks, so far, can only be produced in a manufacturing setting which results in a limited range of sorbent chemistry selection [70]. In addition, cartridges are easier to be scaled up for larger sample loads and better capable of a cleanup than disks. Because of the low selectivity of sorbents and the difficulty of manufacture, there are not many choices of commercial SPE disks in the market that makes disks significantly more costly than cartridges. Although SPE disks require a smaller elution volumes and can be operated at higher flow rates [71].

In SPE, the solid sorbent is usually consisting of chemically bonded silica particles or small particles of an organic polymer resin with pores to enhance the surface area for interaction between the liquid sample and the extractant [72]. Other sorbents also have been developed such as activated carbon, alumina, silica gel, and magnesium silicate [73].
Because of the very polar nature of the bare silica, it is not a good stationary phase for samples with aqueous solvent. Therefore, it needs to be modified to a more hydrophobic sorbent for application to aqueous systems.

SPE can be classified into three major groups based on different modified silicic stationary phases, in which different chemical mechanisms are applied to transfer the analytes from a particular matrix. These three groups are: normal phase, reversed phase, and ion exchange. Sorbent selection is based on considerations of the properties of the solution and the target analytes.

If the analyte has a strong hydrophobic property, a sorbent can be modified to have a hydrophobic surface to separate the analyte. For a reversed phase separation, the cartridges are intended to extract nonpolar to moderately polar compounds from a polar or moderately polar matrix (e.g. water) with a nonpolar stationary phase [74]. The van-der-Waals forces between the bonds in the analyte and the functional groups on the sorbent surface separate the analyte from the polar solutions and the analyte is then retained on the SPE sorbent [67]. A nonpolar solvent is subsequently used to desorb the compound from the sorbent. Typical reversed phase materials include carbon-based media, polymer-based media, polymer-coated, and bonded silica media [67]. C18 cartridges, as the most widely used and traditional reversed phase extraction device, are utilized to partition dissolved organic compounds such as antibiotics, essential oils, drugs, esters, and fat-soluble vitamins from different matrices. Other reversed phase sorbents have also been developed for specific needs.

Normal phase SPE, on the other hand, is typically exploited to extract a polar solute from a mid-polar to nonpolar matrix such as acetone, hexane and chlorinated solvents with a polar stationary phase.

In addition to hydrophobic interaction, ionic interaction between an analyte and the sorbent in aqueous sample matrix can also be utilized. Ion exchange SPE can be used to extract compounds with charges in a solution. Anionic analytes can be attracted to the silica surface bonding with an aliphatic quaternary amine group. Cationic compounds are isolated on an aliphatic sulfonic acid group that is bonded to the silica surface. The electrostatic attraction forces between the charged functional group in the compound and the charged group bonded to the silica surface is the primary retention mechanism of ion exchange SPE [67]. With the further development of SPE technology, mixed-mode sorbent systems that are combinations of reversed phase and ion-exchange sorbent have become available. Some studies have already addressed that mixed-mode sorbents are often advantageous and provide better separation (of target analytes from the matrix) than reversed phase or ion-exchange SPE alone [75, 76].

A typical SPE procedure involves the following steps: 1. Column conditioning; 2. Sample loading; 3. Interference removal, and 4. Analyte elution. This procedure is shown in Figure 1.3.

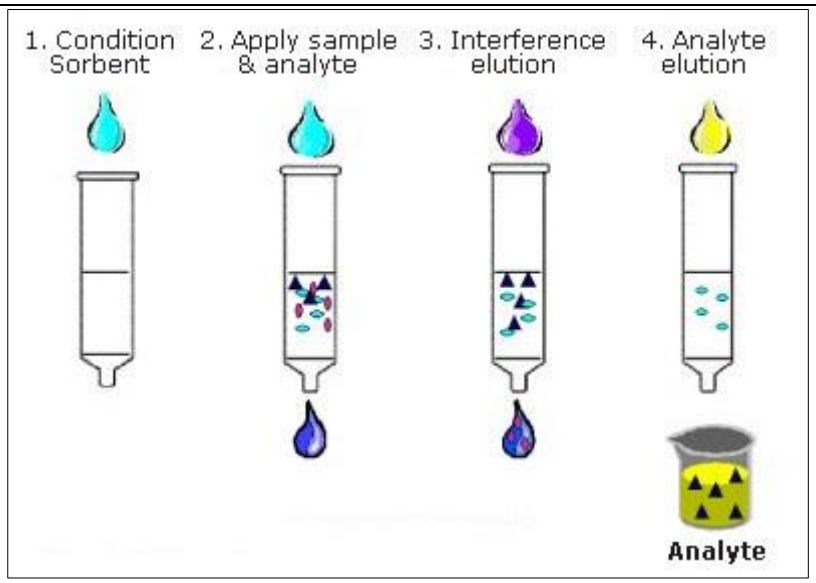

Figure 1.3: Typical SPE procedure for enriching and eluting of analytes from water matrix [77]

First, the modified silica surface needs to be conditioned with an organic solvent such as methanol in order to be active (wetted) and available for the analytes [73]. The purpose of the conditioning step is chain extension. During the extension process, an organic solvent is added to the matrix as a wetting agent to keep the chains fully extended for the interactions between the sorbent and analytes. After that, excess organic solvent is removed from the sorbent by Milli-Q water.

In the second step, the sample containing analytes of interest is loaded onto the column with vacuum. The loading rate is necessary to be adjusted to ensure that the analytes will have enough contact time with the sorbent phase.

An interference removal step usually follows sample loading. In this step, the cartridge would be rinsed with a suitable solvent to remove the interference that may affect accurate determination of the analytes. After that, the cartridge will be left with vacuum open to remove any remaining water. Water would also be considered as interference if water miscible solvents are used.

The final and most important step is elution of the analytes from the sorbent. In order to use minimum volume of elution solvent, an appropriate solvent must be chosen to enhance the interactions between matrix and sorbent or between matrix and analytes, and minimize the interactions between sorbent and analytes. In addition to solvent selection, sufficient contact time between the sorbent and solvent is important to ensure a quantitative removal of the analytes from the sorbent.

A comprehensive literature review was done to show the use of SPE as an enrichment step prior to bio-tests in different aquatic environments as presented in Table 1.1.

Bioassays are used to monitor the quality of water with regard to the presence of certain chemicals that are relevant for a toxic action to human and/or the natural environment.

Simple biological systems are used to simulate the immediate effect of a compound or mixtures of compounds on living organisms [78]. It relies on detecting the response of organisms exposed to micropollutants relative to a control [79]. In contrast to chemical analysis, the results of bioassays reflect biological responses instead of chemical concentrations.

The sample extraction process must be standardized and fully validated as its thoroughness will directly influence the quality of bioassay results.

As can be deduced from Table 1.1, the frequently used SPE material is Oasis HLB (hydrophilic-lipophilic balance). Two approaches (target and non-target) were examined to assess the toxicity of compounds in the final extracts using different in vivo, in vitro and in situ experiments. In target approach, the focus was on the presence of specific analyte(s) in water samples. Different analytical techniques were used for the detection and quantification purposes. The results represented the toxicological effects for each individual compound and/or a group of compounds. Limited number of these studies showed how efficient was the SPE material to enrich such compounds from water. According to non-target approach, toxicity results represented the effects for all substances in the extracts (i.e. mixture effect).

Table 1.1: Overview of SPE and bioassay applications for water quality assessment

SPE material	Toxicological method	Analytical method	Water matrix	Screening approach	Ref.
XAD-2	Salmonella/ Mammalian-Microsome mutagenic Assay	NA	DW	Non-target	[80]
XAD-7	SOS/umu-test	NA	SW	Non-target	[81]
Sep-pakC18	Salmonella typhimurium in umu test assay (genotoxicity)	NA	WWTP	Non-target	[82]
Octadecylsilane (C18)	Yeast-based screen assay for estrogenic activity	GC-MS	WWTP	Target	[83]
ENV+; RP-C18	Estrogen Screen (E-SCREEN) assay	GC-MS	WWTP	Target	[84]
C18	Enzyme-Linked Lmmunosorbent Assay (ELISA)	GC-MS; GC-MS/MS	SW and WWTP	Target	[85]
Octylsilane, Isolute ENV+ and ENVI-Carb in series	Yeast Estrogen Screen (YES) assay	GC-MS	SW	Target	[86]
C18 and ENV+ in series	Daphnia magna bioassay	GC–MS	Stormwater	Target	[87]
SDB-XC	Estrogen receptor (ER)-binding assay, YES assay and ER-mediated chemically activated luciferase gene expression (ER-CALUX) assay	NA	SW and WWTP	Non-target	[88]
RP-C18	In situ hepatic vitellogenin expression from caged rainbow trout (Oncorhynchus mykiss); in vitro bioassays YES, ER- luciferase assay, primary rainbow trout hepatocytes)	GC-Ion trap-MS/MS; LC-MS/MS; GC-MS	SW and WWTP	Target	[89]
Isolute C-18	Acute toxicity: Microtox, and Ceriodaphnids; Chronic toxicity: Algae, Rotoxkit, Fish and Ceriodaphnids	GC-MS	WWTP	Target	[90]
XAD-2	Salmonella/microsome assay (Ames test), the Arabinose resistance test (Ara test) and the SOS/umu test	GC-MS	Tap water	Target	[91]

Table 1.1: Overview of SPE and bioassay applications for water quality assessment (continued)

Sep-Pak Plus tC18	Plant bioassays (Tradescantia/Micronucleus test, Allium cepa test, Vicia faba test); Fish bioassays (Comet test in erythrocytes, Micronucleus test in erythrocytes); Mollusc bioassays (Comet test in hemocytes, Micronucleus test in hemocytes); In vitro tests with bacteria (Ames test, Mutatox, Microtox, SOS Chromotest); In vitro tests with yeast (Saccharomyces cerevisiae); In vitro tests with human cells Micronucleus test in lymphocytes, Comet test in lymphocytes, Enzymatic activity test, Cytotoxicity test); In vitro tests with fish cells (Enzymatic activity test, Cytotoxicity test)	GC-MS	DW	Target	[92]
XAD-8 over XAD-2	Salmonella Microplate Cytotoxicity Assay, Salmonella Preincubation Mutagenicity Assay, Mammalian Cell Microplate Cytotoxicity Assay and Single Cell Gel Electrophoresis (SCGE) Assay	GC-MS	DW	Target	[93]
Mixed LiChrolut RP18 and LiChrolut EN	YES assay and by measuring the blood plasma vitellogenin concentrations in exposed male rainbow trout (Oncorhynchus mykiss)	GC-MS	WWTP	Target	[94]
Mixed LiChrolut RP18 and LiChrolut EN	YES, production of zona radiata proteins in trout hepatocytes, and the induction of reporter gene expression in the transfected rainbow trout gonad cell line	GC-MS	WWTP	Target	[95]
Isolut RP-C18; SPE Isolut C2/ENV+	Microtox test, Daphnia magna and Ceriodaphnia dubia tests	GC-MS; LC-MS	WWTP	Target	[96]
RP-C18	YES bioassay	GC-Ion trap-MS/MS; LC-MS/MS; GC-MS	SW and WWTP	Target	[97]
Sep-Pak Plus tC18	Cytotoxicity assays (Short-term exposure , Long-term exposure, Neutral red uptake assay, Lactate dehydrogenase release assay); Comet assay; Micronuclei assay	NA	DW	Non-target	[98]

Table 1.1: Overview of SPE and bioassay applications for water quality assessment (continued)

Octadecylsilane (C18)	Daphnia magna, Chlorella vulgaris bioassays, Salmonella typhimurium, recombinant yeast screen, and Oryzias latipes embryolarval tests	GC-MS	WWTP	Target	[99]
Oasis HLB	Phytotoxicity assay	LC-MS	SW	Target	[100]
Sep-Pak Plus tC18	Mutagenicity in the Salmonella typhimurium reversion test; genotoxicity assays (the Allium cepa test)	NA	WWTP	Non-target	[101]
Oasis HLB	Algal bioassay	GC-MS	SW and GW	Target	[102]
Oasis HLB	Phytotoxicity assay	LC–MS	SW	Target	[103]
C18-silica	Comet Assay and Micronucleus Assay	NA	SW	Non-target	[104]
Oasis HLB	YES assay	LC-MS/MS	SW	Target	[105]
Sep-Pak Plus tC18	In vitro genotoxicity tests (Salmonella/microsome assay; SOS Chromotest; gene conversion; point mutation and mitochondrial deoxyribonucleic acid (DNA) mutability assays) and for a toxicity test (Microtox)	NA	DW	Non-target	[106]
Supelclean C18 with octadecyl-bonded endcapped silica sorbent; Oasis HLB with n-vinylpyrrolidone and divinylbenzene copolymer sorbent; Isolute C2/C18(EC)	ER-binding assay, a rainbow trout ER-binding assay, E-SCREEN, and a rainbow trout androgen-receptor-binding assay	NA	WWTP	Non-target	[107]
Sep-Pak Vac C18	Daphnia magna test	GC-MS	WWTP	Target	[108]
Oasis; SPE C18	Bioluminescence inhibition tests based on Vibrio fischeri	GC-MS; LC–MS	WWTP	Target	[109]
Oasis HLB	Chlorophyll fluorescence bioassay	LC-MS	SW	Target	[110]
Oasis HLB	In vitro estrogenic equivalent	GC-MS	WWTP	Target	[111]

Table 1.1: Overview of SPE and bioassay applications for water quality assessment (continued)

Oasis HLB	Ethoxyresorufin O-deethylase (EROD) activity, vitellogenin induction (estrogenic activity), cytotoxicity (membrane stability and metabolic inhibition)	GC-MS; LC-MS/MS	WWTP	Target	[112]
C18	Recombinant yeast assay	GC; GC-MS	DWTP	Target	[113]
Sep-Pak Plus tC18	in vitro cytotoxic and genotoxic effects (DNA damage by the comet assay)	NA	DW	Non-target	[114]
Strata-X	Acute toxicity tests using the microbe Vibrio fischeri, freshwater macroinvertebrates Daphnia magna and Moina macrocopa, and fish (Oryzias latipes)	LC-MS/MS	SW and WWTP	Target	[115]
Isolute ENV+ and S-X3	Microtox assay for acute toxicity and YES assay	GC-MS; GC-ECD	SW	Target	[116]
Serdolit PAD-1	Umu short-term genotoxicity test	NA	WWTP	Non-target	[117]
HLB	Phytotoxicity assay	LC-MS	SW	Target	[118]
HZ-802	Cellar bioassay	NA	WWTP	Non-target	[119]
ENV+ and octadecylsilane	Growth inhibition assay	NA	SW	Non-target	[120]
Oasis HLB	Recombinant yeast bioassay	GC-MS	WWTP	Target	[121]
Sep-Pak Plus tC18	Salmonella (Ames) test	NA	WWTP	Non-target	[122]
XAD-8 over XAD-2	Salmonella mutagenicity assay	NA	DW	Non-target	[123]
LiChrolut EN plus LiChrolut RP-C18; Empore SDB-RPS; Empore C18	Inhibition of bacterial luminescence, Inhibition of algal growth, Inhibition of photosynthesis, Inhibition of acetylcholine esterase, YES assay, and Genotoxicity umuC test	NA	SW and WWTP	Non-target	[124]
Oasis HLB	In vitro inflammatory responses	LC–MS/MS	SW	Target	[125]
Sep-Pak Plus tC18	Micronucleus and Comet assays	NA	DW	Non-target	[126]

Table 1.1: Overview of SPE and bioassay applications for water quality assessment (continued)

Oasis HLB	Umu assay, Yeast two-hybrid assay, Daphnia magna bioassay, and Japanese medaka embryo exposure test	NA	WWTP	Non-target	[127]
Oasis HLB	In vitro Cellular bioassays for the evaluation of hyroid and estrogenic activites	LC-MS/MS	SW, DWTP and WWTP	Target	[128]
Empore C18 FF	In vitro transthyretin, thyroid receptor, and luciferase assays	NA	paper manufacturing plants, SW and WWTP	Non-target	[129]
XAD-2	Micronucleus assay; Single cell gel electrophoreses assay	NA	SW	Non-target	[130]
LiChrolut EN plus LiChrolut RP-C18	Bioluminescence inhibition test, combined algae test, YES assay, acetylcholinesterase (AChE) inhibition assay, and umuC assay	NA	WWTP	Non-target	[45]
Oasis HLB	Bioluminescence inhibition test, AChE Inhibition Assay, Imaging-PAM Assay, E-SCREEN, Aryl hydrocarbon receptor-chemically activated fluorescent gene expression (AhR-CAFLUX) and umuC assay	NA	WWTP	Non-target	[131]
Oasis HLB	3 bioassays (fish, Daphnia and algae)	LC-MS/MS	SW	Target	[132]
Oasis HLB	Estrogenic activity (E-SCREEN assay), AhR-CAFLUX, neurotoxicity (AChE inhibition assay), phytotoxicity (PSII inhibition I-PAM assay) and genotoxicity (umuC assay)	LC-MS/MS	WWTP	Target	[133]
Oasis C18	Recombinant yeast assay	NA	WWTP	Non-target	[134]
Oasis HLB	YES, ER-CALUX, MELN, T47DKBluc, and E-SCREEN assays	GC-MS; GC-ECD; LC-MS-MS	SW and GW	Target	[135]
XAD-4	Salmonella/Microsome Microsuspension Assay	NA	SW and industrial effluent water	Non-target	[136]
Suplco	Toxicological tests on Spermatogenic cells, Sertoli cells and Leydig cells of male rats	GC-ECD; GC-MS	SW	Target	[137]

Table 1.1: Overview of SPE and bioassay applications for water quality assessment (continued)

Oasis HLB	Cytotoxicity, chronic toxicity, EROD activity, inhibition of the multixenobiotic resistance, genotoxicicity and estrogenic potential	GC-MS; LC-Q-TOF-MS	WWTP	Both	[138]
Oasis HLB	In vitro bioassays. Yeast-based test (YES; yeast anti estrogen screen; yeast androgen screen; yeast anti androgen screen; yeast dioxin screen), Cytotoxicity assay, Estrogenic activity, Anti-androgenic activity and AhR agonistic activity	NA	WWTP	Non-target	[139]
Oasis C18	In vitro Bioassays (Estrogenic Activity, (Anti)Androgenic Activity (Anti)Progesteronic Activity and (Anti)Thyroidal Activity	NA	WWTP	Non-target	[140]
Oasis HLB	Baseline Toxicity (Bioluminescence inhibition in Vibrio fischeri), Neurotoxicity (AChE), Phytotoxicity (Max-I-PAM), Estrogenicity (E-SCREEN), AhR-CAFLUX and Genotoxicity (UmuC)	NA	Purified recycled water, DWTP and WWTP	Non-target	[141]
Mixed C18-HD, Oasis HLB, Bakerbond SDB[1], SDB[XC], Isolute ENV+, and ENVI-Carb Plus	Estrogenic activity using a human cancer cell line (MCF7, E-SCREEN) bioassays	NA	Bottled mineral water	Non-target	[142]
Octadecyl C18FF	Estrogenic activity (human and medaka estrogen receptor a bioassays) and total estrogens (ELISA)	NA	WWTP, freshwater and estuary	Non-target	[143]
C18	Green monkey kidney fibroblast cell-based thyroid hormone reporter gene assay	GC-ECD; LC-MS/MS; LC-UV	SW	Target	[144]
Oasis HLB	Bioluminescence inhibition test with Vibrio fischeri	LC-Q-LIT-MS	WWTP	Target	[145]
Oasis HLB	YES bioassay	GC-MS	SW	Target	[146]
Oasis HLB	Microtox assay, E-SCREEN, and Photosynthesis inhibition	LC-MS/MS	WWTP	Target	[147]
Oasis HLB	Ames and Comet assays	NA	SW	Non-target	[148]

Table 1.1: Overview of SPE and bioassay applications for water quality assessment (continued)

BAKERBOND Polar Plus C18 (Octadecyl)	In bacteria (Salmonella/microsome assays), in a plant bioassay (micronucleus assay with root tip cells of Allium cepa), and in SCGE tests with mammalian cells	NA	WWTP	Non-target	[149]
Oasis HLB	Thyroid receptor agonistic activity test and thyroid receptor antagonistic activity test (Galactosidase assay)	NA	WWTP	Non-target	[150]
Oasis HLB	ER-CALUX assay	GC-MS	WWTP	Target	[151]
Sep-Pak Plus PS-2	Umu genotoxicity test (using Salmonella typhimurium strain)	NA	SW and WWTP	Non-target	[152]
Oasis HLB	In vitro bioassays (YES, yeast androgen screen, and genotoxicity assay [umu/SOS])	GC-MS	Textile and dyeing plants, electronic and electroplate factories, pulp and paper mills, fine chemical factories, and WWTP	Target	[153]
XAD	In vitro mammalian cell toxicity	GC-MS; GC-TOF-MS	DW	Target	[154]
Chromabond Easy	Mutagenic activity (Ames test) and Genotoxicity (umu test)	LC-MS/MS	DW	Target	[155]
HyperSep C18	YES assay	NA	WWTP	Non-target	[156]
Oasis HLB	In vitro cytotoxicity assays (bacterial cytotoxicity [Microtox], mammalian cell cytotoxicity); Reactive toxicity bioassays (umuC assay for genotoxicity, the Escherichia coli biosensor for reactive toxicity toward proteins, and the AREc32 assay for oxidative stress)	GC-ECD; IC	DWTP	Target	[157]
Oasis HLB	In vitro Escherichia coli growth assay	NA	WWTP	Non-target	[158]
Oasis HLB	Thyroid hormone reporter gene assay based on the green monkey kidney fibroblast	GC-ECD	SW and GW	Target	[159]

Table 1.1: Overview of SPE and bioassay applications for water quality assessment (continued)

Oasis HLB	In vitro bioassays: Nonspecific cytotoxicity (Microtox), specific effect of photosynthesis inhibition, estrogenic activity (ER-CALUX), dioxin-like (AhR-CAFLUX) activity and oxidative stress response (AREc32)	NA	SW	Non-target	[160]
Oasis HLB	Yeast and diatom culture bioassays; Estrogenity and dioxine like activity	LC-MS-MS; GC-MS	WWTP	Target	[161]
Oasis HLB	Daphnia magna assay	GC-MS/MS	SW		[162]
Oasis HLB	Toxicity screening in a series of small scale or in vitro bioassays. The bioassays included determinations of cytotoxicity, EROD activity; inhibition of the multixenobiotic resistance, genotoxicicity and estrogenic potential	GC-MS; LC-Q-TOF-MS	SW and WWTP	Target	[163]
Chromabond HR-X	Growth inhibition test	LC-MS/MS	SW	Target	[164]
Sep-Pak Plus C18	YES assay	LDTD–MS/MS	WWTP	Target	[165]
Oasis HLB	Non-specific toxicity (Microtox and combined algae test), the specific modes of action of phytotoxicity (combined algae test), dioxin-like activity (AhR-CAFLUX), and estrogenicity (E-SCREEN); reactive toxicity encompassing genotoxicity (umuC) and oxidative stress (AREc32)	NA	Stormwater	Non-target	[166]
Oasis MCX	In vitro bioassays (Comet assay (genotoxicity, DNA strand breaks), the Ames fluctuation assay (genotoxicity, gene mutations) and a panel of CALUX assays (endocrine disruption))	LC-LTQ Orbitrap-MS	SW	Target	[167]
Oasis HLB followed by Supelclean coconut charcoal	Microtox bioassay (bioluminescence inhibition in Vibrio fischeri); baseline toxicity; mixture effect	GC-MS; LC-MS	WWTP, recycled water, stormwater, SW and DW	Target	[168]
Oasis HLB	Bioluminescence inhibition assay with Vibrio fischeri (Microtox), umuC, Escherichia coli and induction of oxidative stress response in AREc32	GC-ECD	WWTP	Target	[169]

Table 1.1: Overview of SPE and bioassay applications for water quality assessment (continued)

Oasis HLB	AREc32 bioassay cytotoxicity	GC-MS/MS; LC-MS/MS	WWTP, DW, SW and stormwater	Target	[170]
Oasis HLB	CALUX bioassays	NA	SW	Non-target	[171]
XAD-2	Antioxidant response element-regulated genes and Antioxidant response element-luciferase reporter gene assays	GC-MS; LC-MS/MS	DW	Target	[172]
Oasis HLB	Estrogenic activity by ER-CALUX assay	LC-MS/MS; LC-LTQ Orbitrap-MS	DWTP	Target	[173]
Strata-X	Bioluminescent Microtox test	LC-MS/MS	SW	Target	[174]
Oasis HLB; SPE Strata-X	Growth inhibition test on Pseudokirchneriella subcapitata and the immobilisation test on Daphnia magna	LC-MS; AAS	SW and GW	Target	[175]
Oasis HLB	Genotoxicity and mutagenicity in vitro (UmuC assay, Ames assay and Chronic toxicity); Genotoxicity in vivo (Comet assay)	LC-MS/MS	WWTP	Target	[176]
Oasis HLB and Supelclean coconut charcoal cartridges in series	Primary nonspecific assays (cytotoxicity to various cell types), specific (inhibition of AChE and endocrine receptor-mediated effects) and reactive toxicity (mutagenicity and genotoxicity), as well as markers of adaptive stress response (modulation of cytokine production) and xenobiotic metabolism (liver enzyme induction)	LC-MS/MS; GC-MS/MS; GC-ECD	Recycled water: treated wastewater and product water (reclaimed water)	Target	[177]
XAD-2	In vivo toxicity test (Effects on reproduction, growth, or survival of Moina macrocopa, a freshwater waterflea and Oryzias latipes, Japanese medaka fish)	GC-MS; GC–ECD; ICP-MS	SW	Target	[178]
Oasis HLB	In vitro bioassays: Bacterial toxicity (Microtox), genotoxicity (umuC), photosynthesis inhibition (Max-I-PAM) and endocrine effects (E-SCREEN and AR-CALUX); in situ effects using mosquitofish (Gambusia holbrooki)	LC-MS/MS	WWTP and recycled water	Target	[179]

Table 1.1: Overview of SPE and bioassay applications for water quality assessment (continued)

Oasis HLB followed by Supelclean coconut charcoal	103 bioassays	GC-MS; LC-MS	WWTP, recycled water, stormwater, SW and DW	Target	[180]
SupelClean coconut charcoal and SupelSelect HLB	Vibrio fischeri bioluminescence inhibition assay, IPAM photosynthesis inhibition assay, umuC genotoxicity assay without metabolic activation, umuC genotoxicity assay with rat S9 metabolic activation, and AREc32 oxidative stress response assay	GC-MS/MS, LC-MS/MS	WWTP and an Advanced Water Recycling Plant	Target	[181]
Oasis HLB	Microtox assay, AREc32 assay, umuC assay, and CellSensor p53RE-bla HCT-116 assay	GC-ECD	Swimming pool water	Target	[182]

AAS: Atomic absorption spectrometry; AChE: Acetylcholinesterase; AhR: Aryl hydrocarbon receptor; CAFLUX: Chemically activated fluorescent gene expression; CALUX: Chemically activated luciferase gene expression; DNA: deoxyribonucleic acid; DW: Drinking water; DWTP: Drinking water treatment plant; ECD: Electron capture detector; ELISA: Enzyme-linked lmmunosorbent assay; ER: Estrogen receptor; EROD: Ethoxyresorufin O-deethylase; E-SCREEN: Estrogen Screen; GC: Gas chromatography; GW: Ground water; IC: Ion chromatography; ICP: Inductively coupled plasma; LC: Liquid chromatography; LDTD: Laser diode thermal desorption; LIT: Linear ion trap; LTQ Linear trap quadrupole; MS: Mass spectrometry; MS/MS: Tandem mass spectrometry; NA: Not available; Q: Quadrupole; SCGE: Single cell gel electrophoresis; SW: Surface water; TOF: Time-of-flight; UV: Ultraviolet; WWTP: Wastewater treatment plant; YES: Yeast estrogen screen

The overall objective was the investigation of the occurrence of organic micropollutants and their ozonated TPs in surface water (Ruhr river) and several wastewater samples taken after different treatment processes of Duisburg-Vierlinden WWTP (before ozonation, after ozonation, and final effluent after biological treatment).

The first aim was to develop a proper SPE method that would allow isolation of 25 target analytes with different physicochemical properties from various aqueous samples (Chapter 2). For this, different commercial SPE materials from two manufacturers were tested and other factors like sample pH and washing and elution solvents were optimized. An analytical method was developed and validated for analysis of the selected compounds in tap water, surface water and wastewater samples. The final procedure was then applied to investigate the presence of analytes in surface water and wastewater samples which were collected during two sampling campaigns.

Another aim was to examine the possible occurrence of 245 suspect analytes in surface water and wastewater samples based on their exact mass using the previously developed SPE method (Chapter 3). An automated LC-HRMS method was used for screening operated in a full scan mode using different search criteria. The suspects were tracked in WWTP after each treatment step with regard to their degradation and/or formation and the release afterwards into surface water.

At the end, the major conclusions from the work are summarized and an outlook on further investigations is given (Chapter 4).

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2 Tandem anion and cation exchange solid phase extraction for the enrichment of micropollutants and transformation products from ozonation

2.1 Abstract

The presence of organic micropollutants and their transformation products (TPs) from biotic and abiotic processes in aquatic environments is receiving intense public and scientific attention. Yet a suitable sample preparation method that would enable extraction and enrichment of a wide range of such compounds from water is missing. The focus of this paper was to develop an enhanced solid phase extraction (SPE) protocol which enables isolation of parent compounds and low molecular weight metabolites (that are produced after treatment of water with ozone) from different water matrices. Ten SPE sorbents were evaluated with regard to their ability to extract acidic, neutral and basic compounds from water at several pH values. Highest recoveries (91-99%) for all analytes in pure water were obtained by combining strong anion and cation exchangers of two manufacturers in a tandem mode without pH adjustment. Tandem Oasis (MAX+MCX) was finally applied to extract the spiked analytes from tap water, surface water and several wastewater samples. The efficiency of the used SPE procedure was examined based on developed and optimized liquid chromatography-electrospray ionization-tandem mass spectrometry (LC–ESI-MS/MS) method using multiple reaction monitoring (MRM) mode. Occurrence of some of the investigated TPs in environmental water matrices has been proven for the first time in this study. Method quantification limits (MQLs) for all compounds ranged in all matrices from 3.7 to 15.3 ng/L. Recoveries (%RE) were between 90 and 110%. Intra-day and inter-day precision, expressed as relative standard deviation, varied from 0.7 to 5.9% and 1.8 to 10.3%, respectively. Matrix effect (%ME) evaluation demonstrated that even complex sample matrices did not show significant ion suppression or enhancement. The applicability of the method was shown during two sampling campaigns at Ruhr river and a wastewater treatment plant (WWTP) equipped with a polishing ozonation step after biological treatment. All parent compounds were found in every water matrix at concentrations ranging between low ng/L and low µg/L. Concentration levels of the detected TPs were in the lower ng/L range. Their concentrations increased after ozonation of treated wastewater but decreased substantially after a polishing biological treatment in the final

effluent and in the receiving surface water, thus demonstrating that occurrence at critical concentrations in aquatic ecosystems is rather unlikely.

2.2 Introduction

In recent years, the number of studies on the occurrence and fate of emerging contaminants such as pharmaceuticals, personal care products, industrial chemicals and disinfection by-products in the aquatic environment have increased steadily [1]. Effluents from wastewater treatment plants (WWTPs) constitute one of the most important sources of organic micropollutants released into the environment [2]. The occurrence of a large spectrum of micropollutants in the environment clearly shows that conventional WWTPs are not capable of fully eliminating these compounds [3-5]. In order to reduce pollutant loads in the WW effluents and improve receiving surface water quality, several technologies such as activated carbon adsorption [6-10], ozonation and advanced oxidation processes [11-16], and membrane filtration [17, 18] have been applied and discussed intensively. More specifically, ozone has demonstrated a high effectiveness in the degradation of micropollutants during wastewater treatment [19, 20]. A main drawback of ozonation is that it does not lead to a full mineralization of organic compounds but to the formation of transformation products (TPs), which might be potentially toxic [21]. 2,6-dichloroaniline for example, a toxic TP, has been shown to be formed during ozonation of diclofenac in an aqueous matrix [22].

The need for proper sample preparation techniques is still a challenging task. Up to date, no method specifically aiming at the extraction and enrichment of polar TPs produced during (advanced) wastewater treatment has been reported.

Solid phase extraction (SPE) is one of the most important and frequently used sample preparation techniques for either matrix simplification or trace enrichment, and has replaced classical liquid–liquid extraction to a large extent [23]. SPE offers several benefits such as (i) high recoveries for compounds of interest, (ii) improvements of selectivity, specificity and reproducibility, (iii) potential application to a wide variety of sample matrices, and (iv) use of low solvent volumes during extraction steps [24].

The choice of an appropriate SPE sorbent is the key point because it can control parameters such as affinity, selectivity and capacity. These parameters depend strongly on the interactions between the analytes of interest and the chosen sorbent but also on the type of sample matrix and its interactions with both the analyte and the sorbent [25, 26].

The main aims of this work were: i) evaluation of various SPE sorbents for their ability to extract eight precursor compounds from a variety of therapeutic classes and several of their commercially available TPs from water. In addition to copolymers composed of both hydrophilic and lipophilic monomers, four strong anionic and cationic mixed-mode sorbents, and four weak anionic and cationic mixed-mode sorbents were included in this study. Experiments were also conducted to optimize sample pH and elution solvents; ii) development and validation of an analytical method for simultaneous determination of the selected analytes using liquid chromatography-electrospray ionization-tandem mass spectrometry (LC–ESI-MS/MS). The performance of the method has been evaluated in real waters in terms of linearity, method detection and quantification limits (MDL and MQL), recovery, precision, and the study of matrix effects; iii) applying the developed SPE-LC-ESI-MS/MS method to examine the presence of target compounds in surface waters and several wastewater samples collected at different steps of an advanced treatment processes including ozonation.

2.3 Materials and methods

2.3.1 Chemicals

Methanol, acetonitrile, and water were supplied by Fisher Scientific GmbH (Nidderau, Germany) and were either of HPLC grade or LC-MS grade. Acetone (analytical grade), ammonium hydroxide (30%), ethanol (absolute), ethyl acetate (analytical grade) and formic acid (98-100%) were purchased from Merck (Darmstadt, Germany).

Analyte standards were of high purity (≥97%). Anthranilic acid, p-benzoquinone, 1,2,4-benzenetriol, 1H-benzotriazole, bisphenol A, carbamazepine, catechol, ciprofloxacin, 2,6-dichloroaniline, diclofenac sodium salt, glyoxylic acid monohydrate, hydroquinone, maleic acid, malic acid, malonic acid, metoprolol tartarate salt, cis,cis-muconic acid (c,c-muconic acid), trans,trans-muconic acid (t,t-muconic acid), p-nitrophenol, oxalic acid, oxaloacetic acid, oxamic acid, paracetamol, succinic acid and sulfamethoxazole were purchased from Sigma-Aldrich (Steinheim, Germany).

Physicochemical properties such as the acid dissociation constant (pKa), speciation at pH 7 and the octanol-water Partition Coefficient (log P_{ow}) were predicted using JChem software for Excel, ChemAxon Ltd. (http://www.chemaxon.com) (Table 2.1). The chemical structures of compounds with their pKa values are listed in Table 5.1.

Table 2.1: Compounds uses, molecular mass and physicochemical properties

Compound	Uses	CAS No.	Molecular mass in g/mol	pK_a	Speciation at pH 7	$logP_{ow}$
1H-Benzotriazole	Industry	95-14-7	119.1	0.58, 8.63	neutral	1.30
Bisphenol A	Industry	80-05-7	228.3	9.78, 10.39	neutral	4.04
Catechol	Bisphenol A (TP) [42]	120-80-9	110.1	9.34, 12.79	neutral	1.37
p-Benzoquinone	Bisphenol A (TP) [42]	106-51-4	108.1	-	neutral	1.02
c,c-Muconic acid	Bisphenol A (TP) [42]	1119-72-8	142.1	3.87, 4.65	anionic	0.49
t,t-Muconic acid	Bisphenol A (TP) [42]	3588-17-8	142.1	3.87, 4.65	anionic	0.49
Carbamazepine	Anticonvulsant	298-46-4	236.3	-	neutral	2.77
Anthranilic acid	Carbamazepine (TP) [43]	118-92-3	137.1	1.95, 4.89	anionic	1.45
Glyoxylic acid	Carbamazepine (TP) [43]	298-12-4	74.0	2.61	anionic	-0.13
Oxamic acid	Carbamazepine (TP) [43]	471-47-6	89.1	2.49	anionic	-1.07
Ciprofloxacin	Antibiotic	85721-33-1	331.3	5.76, 8.68	zwitterionic	-0.81
Diclofenac	Analgesic	15307-79-6	296.1	4.00	anionic	4.26
2,6-Dichloroaniline	Diclofenac (TP) [22]	608-31-1	162.0	1.34	neutral	2.35
Metoprolol	Beta blocker	51384-51-1	267.4	9.67	cationic	1.76
Paracetamol	Analgesic	103-90-2	151.2	9.46	neutral	0.91
Oxalic acid	Paracetamol (TP) [44]	144-62-7	90.0	1.36, 4.11	anionic	-0.26
Oxaloacetic acid	Paracetamol (TP) [44]	328-42-7	132.1	2.41, 3.58	anionic	-0.04
Malic acid	Paracetamol (TP) [44]	6915-15-7	134.1	3.2, 5.13	anionic	-1.11
Malonic acid	Paracetamol (TP) [44]	141-82-2	104.1	2.43, 5.92	anionic	-0.33
Maleic acid	Paracetamol (TP) [44]	110-16-7	116.1	3.05, 5.91	anionic	-0.04
Succinic acid	Paracetamol (TP) [44]	110-15-6	118.1	3.55, 5.69	anionic	-0.40
1,2,4-Benzenetriol	Paracetamol (TP) [44]	533-73-3	126.1	9.39, 10.99	neutral	1.06
Hydroquinone	Paracetamol (TP) [44]	123-31-9	110.1	9.68, 11.55	neutral	1.37
Sulfamethoxazole	Antibiotic	723-46-6	253.3	1.97, 6.16	anionic	0.79
p-Nitrophenol	Sulfamethoxazole (TP) [45]	100-02-7	139.1	7.07	neutral	1.61

2.3.2 Sampling and sample preparation

Two sampling campaigns were performed in April, 2014 and February, 2015 to grab water samples.

Sample matrices included (a) 24-h composite wastewater (WW) samples at the municipal wastewater treatment plant (WWTP) located in Duisburg-Vierlinden (Germany) where samples were taken after different treatment steps: before ozonation ($WWBO_3$), after ozonation ($WWAO_3$) and the final effluent after and additional biofilter (WWFE); (b) surface water (SW) from the Ruhr river at Essen-Werden (Germany); and (c) tap water (TW) from the working lab at the University of Duisburg-Essen (campus Essen, Germany).

Grab samples were collected in solvent-rinsed amber glass bottles and stored at 4ºC in the dark in order to minimize degradation. Prior to extraction, the transported samples were filtered with a bottle-top vacuum filtration unit through a glass microfiber filter (GF/F, 0.7 μm average pore size, 47 mm diameter).

Stock solutions at nominal concentrations of 100 μg/mL of each analyte were prepared by dissolving approximately 5 mg in 50 mL HPLC grade methanol or water depending on solubility and stored at 4 ºC in the dark for increased stability. Working solutions, containing the 25 analytes, were prepared by volumetric dilution in water as required from stock solutions and stored at 4 ºC in the dark until use.

2.3.3 SPE procedure

Various sorbents were investigated for sample pretreatment and analyte preconcentration including Oasis HLB, Oasis MAX, Oasis MCX, Oasis WAX, Oasis WCX, all of which were purchased from Waters (Eschborn, Germany), as well as Strata-X, Strata-X-A, Strata-X-C, Strata-X-AW and Strata-X-CW from Phenomenex (Aschaffenburg, Germany). The physicochemical properties of SPE cartridges used are summarized in Table 2.2.

Tandem anion and cation exchange SPE

Table 2.2: Physicochemical properties of SPE catrridges

Sorbent	Particle size (µm)	Pore size (Å)	Surface area (m^2/g)	Sorbent mass (mg)	Cartridge capacity (mL)	Surface modification
Oasis HLB	30	80	810	100, 200	6	Divinylbenzene-*N*-vinylpyrrolidone copolymer
Oasis MAX	30	80	810	100, 200	6	Quaternary amine functionalized divinylbenzene-*N*-vinylpyrrolidone
Oasis MCX	30	80	810	100, 200	6	Sulfonated divinylbenzene-*N*-vinyl-pyrrolidone
Oasis WAX	30	80	810	200	6	Cyclic secondary/tertiary amine functionalized divinylbenzene-*N*-vinylpyrrolidone
Oasis WCX	30	80	810	200	6	Carboxy functionalized divinylbenzene-*N*-vinylpyrrolidone
Strata-X	33	85	800	100, 200	6	Polar functionalized styrene-divinyl-benzene polymer
Strata-X-A	33	85	800	100, 200	6	Quaternary amine functionalized styrene-divinylbenzene polymer
Strata-X-C	33	85	800	100, 200	6	Sulfonated styrene-divinylbenzene polymer with polar surface modification
Strata-X-AW	33	85	800	200	6	Primary and secondary amine functionalized styrene-divinyl-benzene polymer
Strata-X-CW	33	85	800	200	6	Carboxylated styrene-divinyl-benzene polymer

Prior to extraction, cartridges were first preconditioned with 2 x 3 mL methanol followed by equilibration with 2 x 3 mL water. Afterwards, they were connected via large volume adaptors to the sample bottles. One liter ultrapure water samples were spiked with 100 µL standard mixture with a concentration of 500 ng/mL for each compound to yield a final concentration in the sample of 50 ng/L. Subsequently, the samples were adjusted to several pH values (2, 5, 7, 9 and 12) and then passed through the cartridges by vacuum suction (maximum of 65 kPa) at a flow rate of ~ 15 mL/min. After the extraction, the cartridges were rinsed with different organic solvents depending on the kind of sorbent, dried under vacuum for 30 minutes, wrapped in aluminum foil, and stored at -20 ^{o}C until elution. Different washing solvents were utilized based on SPE material type. Oasis HLB and Strata-X were washed with 2 mL of 100% water, while 2 mL of water-ammonia solution (95:5, v/v) mixture was the one for Oasis MAX, Oasis WCX, Strata-X-A and Strata-X-CW. The washing solvent for Oasis MCX, Oasis WAX, Strata-X-C and Strata-X-AW contained 2 mL of water-formic acid (98:2, v/v) mixture.

The elution was assayed with 100% of methanol, 100% of ethyl acetate, and a mixture of methanol-ethyl acetate (70:30, v/v) for both Oasis HLB and Strata-X sorbents. For Oasis MCX, Oasis WAX, Strata-X-C and Strata-X-AW, mixtures of methanol-ammonia solution (95:5, v/v), ethyl acetate-ammonia solution (95:5, v/v), and methanol-ethyl acetate-ammonia solution (67.5:27.5:5, v/v) were tested. Mixtures of methanol-formic acid (98:2, v/v), ethyl acetate-formic acid (98:2, v/v), and methanol-ethyl acetate-formic acid (69:29:2, v/v) were examined for Oasis MAX, Oasis WCX, Strata-X-A and Strata-X-CW. After elution, the eluates were reduced in volume under vacuum before being solvent exchanged to water at a nominal final volume of 1 mL (exact volume determined by weighting the vial).

For further investigation, two SPE cartridges (Oasis MAX & Oasis MCX, 100 mg/6 mL each), (Oasis HLB & Oasis MAX, 100 mg/6 mL each), and (Oasis HLB & Oasis MCX, 100 mg/6 mL each) were conditioned, equilibrated and connected together in a tandem configuration; as well as (Strata-X-A & Strata-X-C, 100 mg/6 mL each), (Strata-X & Strata-X-A, 100 mg/6 mL each), and (Strata-X & Strata-X-C, 100 mg/6 mL each). After enrichment, the cartridges were disconnected and followed the same washing and elution steps for each single one as detailed before. The gathered eluates from both cartridges were combined in a tube and reduced in volume under vacuum with solvent exchange to water as a final solvent until reaching 1 mL.

2.3.4 SPE protocol for real water matrices

200 mg/6 mL Oasis MAX and Oasis MCX cartridges were conditioned and equilibrated with 2 x 3 mL methanol and 2 x 3 mL water respectively. The two cartridges were connected together in a tandem mode in which Oasis MAX was the cartridge connected directly to the sample reservoir while Oasis MCX was the subsequent one. 1 L tap water, SW and WW samples were filtered; left without pH adjustment; and spiked with 100 µL of the respective standard mixture (c = 500 ng/mL). The extraction was carried out on a vacuum manifold via large volume adapters. After drying of the cartridges, the washing and elution were carried out for each cartridge individually. Oasis MAX was washed with 2 mL water-ammonia solution (95:5, v/v) mixture and eluted with 6 mL methanol-ethyl acetate-formic acid (69:29:2, v/v) mixture. The Oasis MCX washing and elution solvents were 2 mL water-formic acid (98:2, v/v) and 6 mL methanol-ethyl acetate-ammonia solution (67.5:27.5:5, v/v) mixtures, respectively. The gathered eluates from both cartridges were mixed together and reduced in volume under vacuum; and the final solvent was changed to 1 mL water.

2.3.5 Mass spectrometry

The samples were analyzed on a liquid chromatograph coupled to a mass spectrometer (LC–MS/MS). The chromatographic separation was performed on an Agilent 1200 system (Agilent Technologies, Waldbronn, Germany) consisting of a binary pump, a vacuum degasser, an autosampler and a thermostated column oven. The HPLC system was coupled to a Sciex API 4000^{TM} mass spectrometer (Applied Biosystems, Darmstadt, Germany) utilizing electrospray ionization (ESI). The mass spectrometer was operated in multiple reaction monitoring (MRM) to achieve the most sensitive and selective detection of the analytes. Each sample was run twice, in positive and negative ionisation mode.

MS/MS parameters were optimized in continuous flow mode, injecting 1000 ng/mL standard solutions at a flow rate of 10 µL/min. Declustering potential (DP), collision energy (CE) and cell exit potential (CXP) parameters were optimized in the auto-tuning program of the Analyst software (Version 1.6.2).

The chromatographic separation was performed on an XSELECT HSS T3 column (150 × 3.0 mm, particle size 3.5 µm, Waters, Germany) and a mobile phase consisting of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The column was eluted isocratically for 7 min with 0% B. Over the following 8 min, the percentage of B was raised to 100% B, kept there for 9 min and finally lowered to 0% B in 1 min. Six min of re-equilibration was allowed prior to the next injection. The flow rate and injection volume were set to 0.3 mL/min and 10 µL, respectively.

2.3.6 Quantification and method validation

Two MRM transitions were monitored for each analyte between the precursor ion and two most abundant fragment ions. The highest characteristic precursor ion/product ion MRM transition was used for quantification purpose, whereas the second one was chosen to confirm the existence of target analytes in the samples. However, the following analytes glyoxylic acid, oxalic acid, malic acid, malonic acid, and maleic acid, exhibited only one MRM transition due to their poor fragmentation.

Quantification was done using eight-point standard addition (n=5) by injecting different analyte masses prepared from the stock standard mixture. The concentration in the sample is obtained using the following equation (Eq. 1):

$$C_{sample} = C_{added} \times \frac{S_{sample}}{S_{sample\ plus\ added} - S_{sample}}$$

where C_{sample} is the initial analyte concentration in the sample, C_{added} is the analyte concentration resulting from the spiked mass in the sample volume, S_{sample} is the signal of the sample, and $S_{sample\ plus\ added}$ is the signal which corresponds to the sample with the spiked standard.

The linearity was estimated by spiking water samples to a final concentration ranging from 20 to 10,000 ng/L. Blank samples (unspiked water samples) were also extracted and used as a quality control, but were not included in the regression analysis.

The method detection limit (MDL) and method quantification limit (MQL) were defined and determined as the lowest observable concentration of analyte from spiked water samples giving a signal-to-noise ratio (*S*/*N*) of 3 and 10, respectively. Both were calculated based on repeated injections (n = 3) of a low level standard.

Recovery (RE) tests were carried out by spiking the analytes at appropriate concentrations in various water samples prior to and after extraction. RE values were evaluated according to the following equation (Eq. 2):

$$\%\ RE = \frac{(P1\text{-}P2)}{(P3\text{-}P2)} \times 100$$

where P1 and P2 are measured peak areas of the analyte in the final extract of spiked and corresponding non-spiked water samples, respectively. P3 is the measured peak area of sample spiked after extraction in the reconstitution step.

Therefore it is essential to study how extracts influence signal response during analysis.

Matrix effects (ME) in the ESI source was determined in different water matrices (TW, SW, $WWBO_3$, $WWAO_3$, and WWFE). ME was calculated using the following equation (Eq. 3) as the percentage of analyte signal suppression or enhancement:

$$\%\ ME = \left[1\text{-}\frac{(P3\text{-}P2)}{P4}\right] \times 100$$

Where P2 and P3 are as described in Eq. 2, and P4 is the peak area of the analyte in the external standard (spiking solution).

The signal of the analyte is suppressed if ME<100 %, whereas the signal of analyte is enhanced if ME>100 %. An ME of 100 % indicates no matrix effect.

To ensure a correct quantification of analytes, method precision and accuracy expressed as relative standard deviation (%RSD), was obtained from the repeated injections (seven-fold) of an extracted

spiked water samples with a concentration in the middle of the linear range, and analyzed during the same day (repeatability/intra-day) and on different days (reproducibility/inter-day).

2.4 Results and discussion

2.4.1 LC-MS/MS performance

Good chromatographic separation of the compounds under investigation was achieved using XSELECT HSS T3 LC column. A series of different mobile phases including methanol and acetonitrile as the organic modifier and water with added formic acid were investigated. Simple gradients of acetonitrile with 0.1% formic acid in both the aqueous and organic phases gave satisfactory separation of the 25 analytes.

The chromatographic setup resulted in sharp peaks with baseline widths generally below 30 s (see Fig. 5.1). Retention times (RT) were between 4.6 and 22.7 min. RT shifts within a sequence were generally lower than 30 s.

Mass spectrometry parameters were optimized by direct infusion of standards for each analyte individually. ESI was used as the ionization source in both negative and positive ion mode by injecting the final extract twice. Detection of the negative precursor ion $[M-H]^-$ was performed for 12 compounds, whereas detection of the positive precursor ion $[M+H]^+$ was performed for the other 13 compounds of interest. Precursor and product ions, collision energies, declustering potential and collision cell exit potential were determined under MS/MS conditions and are summarized in Table 2.3.

Table 2.3: Retention times and parameters for LC-MS/MS monitoring

Compound	Retention time (min)	Precursor ion (m/z)	Product ion (m/z)	DP (V)	CE (V)	CXP (V)
Glyoxylic acid	4.6	73.1	67.8	-50	-24	-5
			–	–	–	–
Oxalic acid	5.9	88.6	43.0	-30	-20	-1
			–	–	–	–
1,2,4-Benzenetriol	6.3	125.2	107.0	-55	-16	-5
			69.0	-55	-22	-5
Oxamic acid	7.4	89.6	82.0	41	49	4
			56.0	41	33	8
Hydroquinone	8.6	111.0	82.0	56	33	4
			65.0	56	27	10
Oxaloacetic acid	9.5	133.3	91.1	121	25	6
			65.0	121	45	2
Paracetamol	10.0	152.2	110.2	51	23	6
			65.1	51	43	10
p-Benzoquinone	10.6	109.2	81.1	71	19	4
			51.2	71	37	8
Malic acid	11.1	132.7	114.8	-45	-16	-1
			–	–	–	–
Catechol	12.0	109.2	91.0	-60	-28	-5
			62.8	-60	-34	-1
Malonic acid	12.8	102.7	41.1	-30	-30	-5
			–	–	–	–
1H-Benzotriazole	13.5	119.9	91.9	51	25	8
			64.9	51	33	4
Maleic acid	14.0	117.1	71.0	-30	-14	-11
			–	–	–	–
Ciprofloxacin	14.9	332.1	288.2	41	23	6
			244.9	41	31	4
Succinic acid	15.7	117.0	99.0	-30	-10	-3
			73.0	-30	-15	-5
Metoprolol	16.3	268.0	116.0	76	27	10
			74.0	76	35	6
t,t-Muconic acid	17.2	141.1	97.1	-40	-12	-5
			53.2	-40	-16	-1
Sulfamethoxazole	17.8	253.9	188.0	66	23	12
			155.8	66	21	14
c,c-Muconic acid	18.6	141.1	97.2	-30	-10	-5
			53.1	-30	-18	-1
p-Nitrophenol	19.0	138.1	107.8	-50	-24	-7
			50.2	-50	-58	-1
Carbamazepine	19.5	236.9	193.9	71	27	16
			179.1	71	49	12
Bisphenol A	20.4	229.3	107.0	26	35	6
			77.2	26	61	4

Table 2.3: Retention times and parameters for LC-MS/MS monitoring (continued)

2,6-Dichloroaniline	21.1	163.2	90.9	61	33	14
			57.1	61	57	8
Anthranilic acid	21.8	138.1	120.0	36	17	10
			92.0	36	29	6
Diclofenac	22.7	293.8	249.8	-50	-28	-15
			213.9	-50	-16	-7

2.4.2 Choice of SPE material

SPE efficiency is linked to a large number of parameters such as the selection of a proper sorbent, enrichment flow rate, pH adjustment, and the composition of washing and elution solvents used in each step of the procedure [28, 29].

The selection of the most adequate SPE sorbent is one of the most important and time-consuming aspects of the method. The widely used Oasis HLB (hydrophilic-lipophilic balance) material, which provides hydrophilic (*N*-vinyl-pyrrolidone) and lipophilic (divinylbenzene-rings) groups for retention of polar and non-polar compounds, is one of the most used sorbent with enormous potential for the extraction of compounds with high polarity [30]. Strata-X is also one of the widely used materials which was included in this study. This material provides lipophilic and hydrophilic sorption properties via a backbone polydivinylbenzene resin containing piperidone groups. Consequently, in this work, several SPE cartridges have been compared and evaluated with regard to Oasis HLB and Strata-X as common baseline materials. The ten tested SPE cartridges contained the same amount of sorbent (200 mg) but differed in their retention nature (see Table 2.2).

To cover the enrichment of ionic hydrophilic compounds, mixed mode sorbents containing ion-exchange groups were added to the SPE material. The anion exchange materials Oasis MAX, Oasis WAX, Strata-X-A and Strata-X-AW were selected to target anionic compounds as well as hydrophilic and lipophilic components. Oasis MCX, Oasis WCX, Strata-X-C and Strata-X-CW were chosen to enrich hydrophilic, lipophilic, and positively charged compounds.

Different elution solvents were assayed for each SPE material at neutral sample pH (see Tables 5.2 and 5.3). Methanol-ethyl acetate (70:30, v/v) was the solvent selected for eluting compounds from Oasis HLB and Strata-X sorbents. A combination of methanol-ethyl acetate-formic acid (69:29:2, v/v) was chosen to elute compounds from Oasis MAX, Oasis WCX, Strata-X-A and Strata-X-CW cartridges, whereas a mixture of methanol-ethyl acetate-ammonia solution (67.5:27.5:5, v/v) was used as an eluting solvent for Oasis MCX, Oasis WAX, Strata-X-C and Strata-X-AW. For the tandem Oasis (MAX+MCX), Oasis (HLB+MAX), Oasis (HLB+MCX),

Strata (X-A+X-C), Strata (X+X-A) and Strata (X+X-C) SPE modes, both washing and elution steps were done for each cartridge separately.

Several papers reported a sample pH adjustment prior to extraction with values ranging from acidic to alkaline pH levels [29]. In this study, several pH values were assayed and varied recovery results were obtained for each extraction material (See Tables 5.4-5.8). However, concluding from these results, pH effects for most compounds were surprisingly low. Thus, samples were processed without pH adjustment in following experiments.

The overall recoveries for all sorbents in pure water at optimized elution solvents and sample pH are listed in Table 2.4. As it can be seen, there are great differences in the retention of analytes among the SPE cartridges. Oasis HLB provided recoveries in the range between 61% for oxalic acid and 75% for hydroquinone. Strata-X achieved recoveries in the range between 60% for glyoxylic acid and 78% for anthranilic acid. The weak anionic and cationic exchangers, (Oasis WAX, Strata-X-AW) and (Oasis WCX, Strata-X-CW), showed unsatisfying recoveries for most compounds. Oasis MAX, Oasis MCX, Strata-X-A and Strata-X-C presented moderate recoveries for all compounds but still did not reach satisfactory values of $\geq$ 90%.

Tandem anion and cation exchange SPE

Table 2.4: Comparison of analyte recoveries (%) and RSD (n=3) obtained on different SPE sorbents from the analysis of spiked pure water samples (without pH adjustement) at optimized elution solvents

Compound	Oasis HLB	Oasis MAX	Oasis MCX	Oasis WAX	Oasis WCX	Strata-X	Strata-X-A	Strata-X-C	Strata-X-AW	Strata-X-CW
1H-Benzotriazole	71 ± 4	75 ± 3	72 ± 2	56 ± 2	50 ± 1	74 ± 2	72 ± 5	72 ± 4	59 ± 2	53 ± 3
Bisphenol A	72 ± 3	74 ± 1	77 ± 4	58 ± 4	45 ± 3	76 ± 1	73 ± 2	74 ± 6	56 ± 1	50 ± 2
Catechol	70 ± 5	72 ± 4	75 ± 2	59 ± 3	54 ± 1	77 ± 4	73 ± 4	72 ± 2	55 ± 5	51 ± 4
p-Benzoquinone	71 ± 2	76 ± 3	74 ± 3	56 ± 5	51 ± 2	76 ± 3	77 ± 1	73 ± 5	58 ± 3	55 ± 5
c,c-Muconic acid	67 ± 2	77 ± 1	70 ± 2	56 ± 2	43 ± 3	63 ± 5	79 ± 3	71 ± 1	59 ± 4	48 ± 1
t,t-Muconic acid	65 ± 5	80 ± 5	65 ± 4	55 ± 4	46 ± 2	62 ± 1	78 ± 2	70 ± 3	58 ± 2	43 ± 3
Carbamazepine	72 ± 3	75 ± 2	75 ± 5	48 ± 3	43 ± 4	76 ± 2	76 ± 5	71 ± 2	44 ± 4	40 ± 2
Anthranilic acid	73 ± 1	80 ± 4	73 ± 3	54 ± 1	48 ± 2	78 ± 4	78 ± 3	67 ± 1	58 ± 1	50 ± 4
Glyoxylic acid	63 ± 4	81 ± 3	63 ± 2	51 ± 3	47 ± 4	60 ± 3	77 ± 2	68 ± 4	46 ± 5	43 ± 5
Oxamic acid	69 ± 3	76 ± 1	66 ± 4	53 ± 5	50 ± 3	66 ± 1	80 ± 6	72 ± 2	56 ± 3	47 ± 1
Ciprofloxacin	74 ± 2	80 ± 2	77 ± 4	59 ± 4	48 ± 2	72 ± 2	78 ± 4	80 ± 5	52 ± 2	45 ± 3
Diclofenac	70 ± 3	76 ± 3	71 ± 1	54 ± 1	52 ± 3	70 ± 1	80 ± 1	73 ± 4	57 ± 5	49 ± 2
2,6-Dichloroaniline	72 ± 1	71 ± 5	75 ± 3	55 ± 4	49 ± 5	76 ± 2	71 ± 3	72 ± 1	52 ± 2	47 ± 1
Metoprolol	65 ± 2	73 ± 2	68 ± 2	53 ± 5	51 ± 1	69 ± 4	70 ± 2	81 ± 2	49 ± 4	48 ± 3
Paracetamol	70 ± 4	72 ± 4	77 ± 3	52 ± 3	50 ± 2	74 ± 5	72 ± 5	72 ± 6	50 ± 2	48 ± 4
Oxalic acid	61 ± 1	78 ± 2	65 ± 3	55 ± 2	53 ± 4	64 ± 2	79 ± 2	68 ± 4	59 ± 3	50 ± 2
Oxaloacetic acid	64 ± 3	78 ± 3	67 ± 2	54 ± 4	52 ± 3	65 ± 5	77 ± 6	71 ± 1	51 ± 1	49 ± 5
Malic acid	62 ± 2	79 ± 1	62 ± 1	58 ± 3	50 ± 4	66 ± 2	80 ± 3	70 ± 3	54 ± 3	53 ± 2
Malonic acid	65 ± 5	77 ± 5	68 ± 3	53 ± 1	48 ± 2	64 ± 1	76 ± 5	73 ± 5	55 ± 4	51 ± 4
Maleic acid	64 ± 3	78 ± 2	67 ± 2	57 ± 5	53 ± 4	65 ± 5	80 ± 2	71 ± 2	54 ± 1	50 ± 3
Succinic acid	70 ± 2	77 ± 3	73 ± 3	55 ± 2	52 ± 3	69 ± 3	75 ± 4	75 ± 3	58 ± 5	55 ± 1
1,2,4-Benzenetriol	71 ± 4	74 ± 2	77 ± 1	49 ± 2	46 ± 5	75 ± 6	76 ± 1	72 ± 1	53 ± 3	51 ± 4
Hydroquinone	75 ± 1	73 ± 4	78 ± 2	53 ± 4	51 ± 3	77 ± 4	76 ± 2	71 ± 5	49 ± 4	48 ± 2
Sulfamethoxazole	72 ± 4	78 ± 1	74 ± 2	57 ± 3	53 ± 2	71 ± 1	78 ± 5	73 ± 4	53 ± 1	49 ± 2
p-Nitrophenol	70 ± 2	75 ± 3	72 ± 1	55 ± 1	52 ± 3	72 ± 2	74 ± 3	71 ± 5	50 ± 5	46 ± 3

From the comparison of ten tested single SPE cartridges, it can be observed that no material has retained all compounds with high recovery yield. Therefore, it was concluded that combining two materials was required.

Strong anionic and cationic exchangers yielded better recoveries compared with weak exchangers. These results were the inspiration to use Oasis MAX in combination with Oasis MCX; and Strata-X-A in combination with Strata-X-C; because this will provide hydrophilic-lipophilic-anionic-cationic interactions. The two ionic exchange materials were therefore used in a tandem mode to investigate recoveries for acidic, basic and neutral compounds. Oasis MAX was chosen as the first SPE material in flow direction to cover the ionic interactions of negatively charged compounds as

well as uncharged hydrophilic and lipophilic compounds. Oasis MCX was used as the second SPE material to extract the positively charged compounds and also the rest of compounds that are not covered by the Oasis MAX material. To achieve a fair comparison between tandem and single SPE strategies, 100 mg of each Oasis MAX and Oasis MCX materials was used to obtain a total of 200 mg in a tandem mode as for single cartridges. The resulting developed SPE method is outlined in Fig. 2.1.

Within this configuration, high recoveries (≥ 91%) were obtained for all compounds without exception as shown in Table 2.5. To further support the idea of simply leaving water samples without pH adjustment, several pH values were tested on the developed SPE tandem approach for both Oasis and Strata materials and recoveries ≥ 90% were obtained for all compounds (see Table 5.9). The acquired data showed that there is no significant effect on recoveries using the tandem SPE approach by changing the pH value of water samples. Recoveries with this approach are superior to previously reported values for a few of the listed parent compounds using different sorbents. Gatidou et al. [31] showed, for example, poor extraction recovery (< 5%) for bisphenol A from wastewater samples using Isolute ENV+ sorbent. Additionally, Weigel et al. [32] showed low recoveries (38-50%) from spiked tap water samples for diclofenac, paracetamol, and metoprolol with extraction using Isolute ENV+. Sacher et al. [33] reported poor recovery for sulfamethoxazole (21%) and moderate recovery (74%) for carbamazepine in SW samples using RP-C18 extraction material. Tuc Dinh et al. [34] presented extraction recovery of 74% for ciprofloxacin from river water using C18 HD cartridges. Liu et al. [35] reported poor recovery (34%) for 1H-benzotriazole in spiked tap water samples using ENVI-18 as an extraction sorbent.

Tandem anion and cation exchange SPE

Table 2.5: Comparison of analyte recoveries (%) and RSD (n=3) in different SPE tandem combinations from the spiked pure water samples (without pH adjustement) at optimized elution solvents

Compound	Tandem Oasis (MAX+MCX)	Tandem Oasis (HLB+MAX)	Tandem Oasis (HLB+MCX)	Tandem Strata (X-A+X-C)	Tandem Strata (X+X-A)	Tandem Strata (X+X-C)
1H-Benzotriazole	96 ± 3	81 ± 4	83 ± 6	92 ± 5	85 ± 4	88 ± 3
Bisphenol A	92 ± 2	84 ± 3	79 ± 3	98 ± 2	81 ± 6	76 ± 2
Catechol	97 ± 4	85 ± 5	82 ± 4	94 ± 5	80 ± 3	78 ± 4
p-Benzoquinone	95 ± 3	85 ± 2	80 ± 3	96 ± 4	87 ± 2	83 ± 6
c,c-Muconic acid	93 ± 5	78 ± 6	81 ± 5	95 ± 2	75 ± 4	85 ± 5
t,t-Muconic acid	94 ± 3	77 ± 6	75 ± 3	92 ± 4	73 ± 3	72 ± 4
Carbamazepine	96 ± 6	85 ± 3	81 ± 6	91 ± 6	88 ± 5	84 ± 2
Anthranilic acid	95 ± 2	83 ± 5	78 ± 2	96 ± 5	80 ± 2	75 ± 5
Glyoxylic acid	92 ± 4	82 ± 3	73 ± 4	94 ± 3	81 ± 6	76 ± 3
Oxamic acid	93 ± 5	80 ± 2	75 ± 5	95 ± 4	84 ± 2	80 ± 4
Ciprofloxacin	96 ± 4	86 ± 5	88 ± 3	91 ± 3	82 ± 4	85 ± 2
Diclofenac	99 ± 3	84 ± 4	82 ± 3	94 ± 5	83 ± 5	80 ± 3
2,6-Dichloroaniline	97 ± 5	83 ± 2	80 ± 4	95 ± 3	79 ± 3	77 ± 6
Metoprolol	92 ± 4	79 ± 3	84 ± 2	99 ± 2	76 ± 3	81 ± 2
Paracetamol	97 ± 2	84 ± 5	80 ± 3	94 ± 5	89 ± 4	84 ± 3
Oxalic acid	95 ± 3	87 ± 6	81 ± 5	93 ± 4	80 ± 6	76 ± 5
Oxaloacetic acid	92 ± 3	89 ± 4	76 ± 2	95 ± 3	84 ± 5	73 ± 4
Malic acid	94 ± 6	86 ± 2	80 ± 3	92 ± 6	81 ± 3	77 ± 2
Malonic acid	95 ± 5	81 ± 4	78 ± 6	92 ± 3	85 ± 5	82 ± 4
Maleic acid	91 ± 4	85 ± 6	79 ± 2	95 ± 5	82 ± 4	74 ± 5
Succinic acid	95 ± 5	87 ± 3	82 ± 4	90 ± 3	83 ± 3	80 ± 3
1,2,4-Benzenetriol	93 ± 3	84 ± 5	83 ± 2	92 ± 2	80 ± 2	78 ± 6
Hydroquinone	95 ± 2	82 ± 3	87 ± 3	90 ± 6	85 ± 4	89 ± 3
Sulfamethoxazole	98 ± 6	80 ± 4	78 ± 2	95 ± 4	77 ± 3	74 ± 4
p-Nitrophenol	92 ± 5	84 ± 3	80 ± 6	94 ± 5	88 ± 2	83 ± 6

For further comparisons, tandem Oasis (HLB+MAX), Oasis (HLB+MCX), Strata (X+X-A) and Strata (X+X-C) were studied and the results showed that these combinations gave better recoveries than each single cartridge but still lower than those obtained by using a combination of strong anionic and cationic exchangers as listed in Table 2.5.

Both tested SPE materials (Oasis and Strata) gave comparable recovery results. In the further study of matrix influences, Oasis materials were used with the tandem approach.

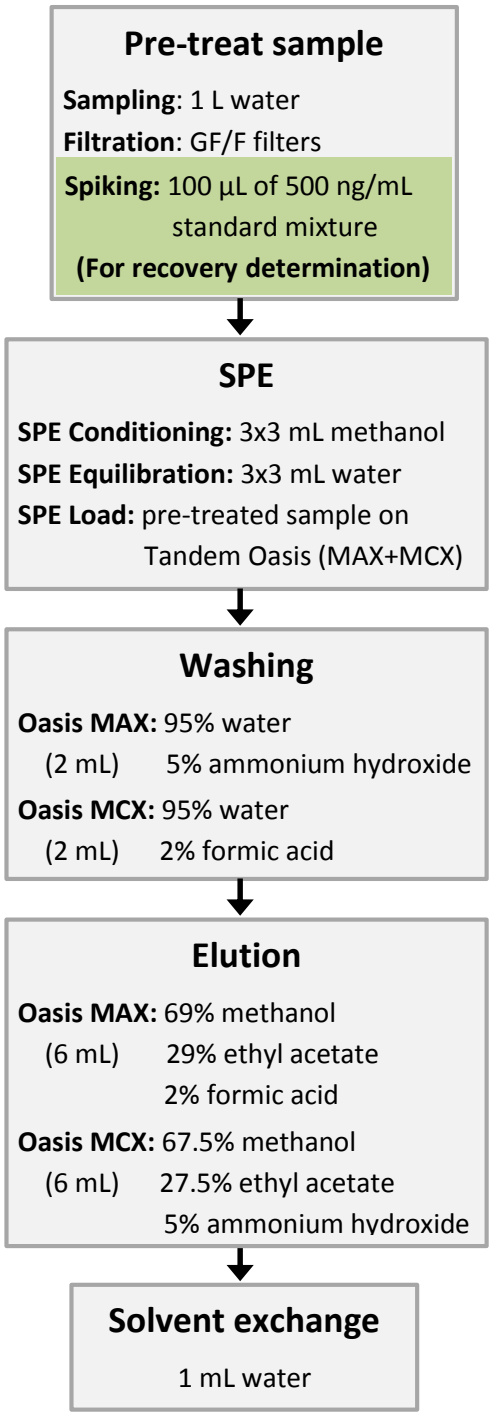

Figure 2.1: Schematic representation of the optimized sample preparation procedure

2.4.3 Method validation

The performance characteristics of the SPE–LC–MS/MS method were established by validation with spiked water samples. Linearity, MDL and MQL, precision, recovery and matrix effect were evaluated for quantitative purposes. The linearity of each analyte was assessed in $WWBO_3$ samples and the mean correlation coefficients (R^2) were higher than 0.9926 thus confirming the

linearity of the method in spite of a very complex matrix (see Table 2.6). To demonstrate the flexibility of the analytical procedure, calibration curves in the same concentration range were also constructed in TW, SW and the rest of WW samples. Excellent linearities with correlation coefficients > 0.99 were obtained for all analytes in all matrices.

MDL and MQL values for the analytes in various matrices are presented in Table 2.6. It can be seen that the MDL and MQL values were the lowest in TW, followed by SW, WWFE, $WWAO_3$, and finally $WWBO_3$. This is probably due to the matrix effects impact.

Recoveries (n=3) were very satisfying ($\geq$ 90%) for all analytes in different water matrices as shown in Table 2.7.

Nowadays mass spectrometry (MS) is largely used in environmental analysis due to its specificity and sensitivity. However, interferences are problems produced, mainly when electrospray is used in the ionization source. The presence of less volatile compounds, which can change the efficiency of droplet formation or evaporation, results in Ion suppression/enhancement. This in turn affects the amount of charged ions in the gas [36, 37]. The evaluation of ME is important to provide accurate and reproducible quantitative data. Table 2.7 summarizes ME on the analytes in various environmental matrices.

A slight signal enhancement was observed in $WWBO_3$, $WWAO_3$, and WWFE for several analytes (bisphenol A, c,c-muconic acid, t,t-muconic acid, glyoxylic acid, oxamic acid, 2,6-dichloroaniline, oxalic acid, malonic acid, maleic acid, 1,2,4-benzenetriol and p-nitrophenol). On the other hand, small ion suppression effects due to matrix constituents were also observed for the rest of compounds. No ME was observed for malic acid in both TW and SW. The influence of the matrix was negligible for all analytes in TW and SW. $WWBO_3$ yielded a slightly higher matrix effect, followed by $WWAO_3$, and then WWFE.

The developed method was sufficiently precise for quantitative analysis of selected compounds in all water matrices. The results obtained are listed in Table 2.8, and showed that the methodology gave good precisions in all water samples even at low concentrations.

Table 2.6: Linearity, method detection and quantitation limits in different water matrices

Compound	Linearity (R^2)	MDL (ng/L)					MQL (ng/L)				
		TW	SW	$WWBO_3$	$WWAO_3$	WWFE	TW	SW	$WWBO_3$	$WWAO_3$	WWFE
1H-Benzotriazole	0.9963	1.1	1.7	2.6	2.3	2.0	3.7	5.7	8.7	7.7	6.7
Bisphenol A	0.9992	1.5	1.8	2.7	2.0	1.9	5.0	6.0	9.0	6.7	6.3
Catechol	0.9926	2.0	2.4	3.0	2.7	2.5	6.7	8.0	10.0	9.0	8.3
p-Benzoquinone	0.9934	2.4	2.5	3.3	3.0	2.9	8.0	8.3	11.0	10.0	9.7
c,c-Muconic acid	0.9981	1.8	2.1	2.8	2.5	2.4	6.0	7.0	9.3	8.3	8.0
t,t-Muconic acid	0.9957	2.0	2.2	2.7	2.4	2.3	6.7	7.3	9.0	8.0	7.7
Carbamazepine	0.9988	1.3	1.5	2.4	2.2	1.8	4.3	5.0	8.0	7.3	6.0
Anthranilic acid	0.9950	1.9	2.3	3.0	2.6	2.5	6.3	7.7	10.0	8.7	8.3
Glyoxylic acid	0.9942	3.0	3.3	4.3	3.9	3.7	10.0	11.0	14.3	13.0	12.3
Oxamic acid	0.9979	2.6	2.8	3.9	3.6	3.2	8.7	9.3	13.0	12.0	10.7
Ciprofloxacin	0.9991	1.5	1.8	2.5	2.3	2.0	5.0	6.0	8.3	7.7	6.7
Diclofenac	0.9984	1.4	1.9	2.3	2.2	2.0	4.7	6.3	7.7	7.3	6.7
2,6-Dichloroaniline	0.9946	2.0	2.4	3.6	3.3	3.1	6.7	8.0	12.0	11.0	10.3
Metoprolol	0.9978	1.8	2.1	3.1	2.8	2.5	6.0	7.0	10.3	9.3	8.3
Paracetamol	0.9983	1.9	2.0	2.8	2.5	2.3	6.3	6.7	9.3	8.3	7.7
Oxalic acid	0.9971	2.5	2.9	4.4	4.2	3.7	8.3	9.7	14.7	14.0	12.3
Oxaloacetic acid	0.9958	2.7	3.1	4.6	4.0	3.5	9.0	10.3	15.3	13.3	11.7
Malic acid	0.9939	2.8	3.0	4.2	3.9	3.8	9.3	10.0	14.0	13.0	12.7
Malonic acid	0.9940	2.5	2.8	4.6	4.1	3.3	8.3	9.3	15.3	13.7	11.0
Maleic acid	0.9965	2.1	2.5	4.2	3.6	3.1	7.0	8.3	14.0	12.0	10.3
Succinic acid	0.9982	1.9	2.2	3.4	3.0	2.6	6.3	7.3	11.3	10.0	8.7
1,2,4-Benzenetriol	0.9951	2.2	2.7	4.0	3.7	3.4	7.3	9.0	13.3	12.3	11.3
Hydroquinone	0.9968	2.4	2.5	4.1	3.7	3.2	8.0	8.3	13.7	12.3	10.7
Sulfamethoxazole	0.9976	1.3	1.5	2.9	2.6	2.3	4.3	5.0	9.7	8.7	7.7
p-Nitrophenol	0.9954	2.4	2.6	4.3	3.5	3.0	8.0	8.7	14.3	11.7	10.0

TW: Tap water; SW: Surface water; $WWBO_3$: Wastewater before ozonation; $WWAO_3$: Wastewater after ozonation; WWFE: Wastewater final effluent

Table 2.7: Recovery (%RE) and matrix effect (%ME) in water samples obtained by applying tandem Oasis (MAX+MCX) SPE mode

Compound	%RE (n=3)					%ME				
	TW	SW	$WWBO_3$	$WWAO_3$	WWFE	TW	SW	$WWBO_3$	$WWAO_3$	WWFE
1H-Benzotriazole	94 ± 5	93 ± 4	95 ± 2	107 ± 5	99 ± 2	99 ± 3	97 ± 6	87 ± 3	89 ±4	93 ± 1
Bisphenol A	93 ± 4	98 ± 3	101 ± 4	102 ± 7	94 ± 3	101 ± 5	103 ± 1	109 ± 5	106 ± 3	104 ± 6
Catechol	95 ± 3	93 ± 2	92 ± 3	94 ± 2	105 ± 5	99 ± 5	96 ± 2	90 ± 5	92 ± 1	95 ± 4
p-Benzoquinone	99 ± 5	92 ± 5	95 ± 4	92 ± 2	90 ± 4	97 ± 2	95 ± 5	88 ± 2	90 ± 5	94 ± 3
c,c-Muconic acid	96 ± 6	103 ± 5	97 ± 2	93 ± 5	90 ± 3	105 ± 6	102 ± 2	112 ± 6	108 ± 3	107 ± 5
t,t-Muconic acid	102 ± 3	93 ± 4	92 ± 3	97 ± 4	102 ± 5	101 ± 3	105 ± 3	115 ± 3	113 ± 4	109 ± 1
Carbamazepine	96 ± 3	94 ± 5	104 ± 5	96 ± 6	98 ± 2	98 ± 1	96 ± 1	85 ± 1	89 ± 2	92 ± 4
Anthranilic acid	97 ± 3	90 ± 2	90 ± 3	94 ± 4	95 ± 6	95 ± 4	94 ± 5	87 ± 4	90 ± 1	91 ± 2
Glyoxylic acid	91 ± 2	92 ± 6	91 ± 5	93 ± 2	90 ± 5	103 ± 2	105 ± 1	114 ± 2	112 ± 2	108 ± 3
Oxamic acid	92 ± 1	94 ± 2	105 ± 4	92 ± 5	102 ± 4	107 ± 3	111 ± 3	111 ± 3	115 ± 4	114 ± 1
Ciprofloxacin	98 ± 3	97 ± 4	96 ± 2	104 ± 4	93 ± 2	99 ± 2	97 ± 2	91 ± 2	93 ± 5	94 ± 3
Diclofenac	95 ± 4	93 ± 2	93 ± 4	101 ± 5	95 ± 3	94 ± 4	93 ± 5	87 ± 4	88 ± 2	91 ± 4
2,6-Dichloroaniline	91 ± 2	108 ± 3	94 ± 3	98 ± 5	91 ± 2	106 ± 3	109 ± 4	115 ± 3	114 ± 1	111 ± 5
Metoprolol	92 ± 1	96 ± 3	103 ± 6	97 ± 2	110 ± 5	95 ± 1	92 ± 6	85 ± 1	87 ± 3	91 ± 2
Paracetamol	101 ± 6	97 ± 1	106 ± 4	96 ± 3	94 ± 2	98 ± 5	95 ± 1	88 ± 5	92 ± 2	93 ± 3
Oxalic acid	92 ± 4	91 ± 3	90 ± 5	93 ± 5	103 ± 6	101 ± 1	104 ± 3	113 ± 1	112 ± 5	109 ± 2
Oxaloacetic acid	95 ± 2	96 ± 2	94 ± 5	91 ± 4	92 ± 4	97 ± 4	93 ± 4	84 ± 4	87 ± 6	89 ± 1
Malic acid	98 ± 5	91 ± 4	91 ± 3	93 ± 5	106 ± 3	100 ± 5	100 ± 2	89 ± 5	93 ± 2	95 ± 4
Malonic acid	90 ± 3	101 ± 3	95 ± 4	92 ± 3	93 ± 5	102 ± 2	106 ± 2	114 ± 2	110 ± 4	107 ± 5
Maleic acid	93 ± 4	95 ± 5	92 ± 3	103 ± 4	90 ± 3	105 ± 4	109 ± 5	116 ± 4	114 ± 1	113 ± 3
Succinic acid	91 ± 5	108 ± 3	96 ± 2	105 ± 2	92 ± 4	97 ± 3	96 ± 3	89 ± 3	90 ± 5	92 ± 1
1,2,4-Benzenetriol	92 ± 2	97 ± 3	90 ± 4	94 ± 5	93 ± 2	101 ± 1	104 ± 5	112 ± 1	107 ± 4	105 ± 2
Hydroquinone	96 ± 4	95 ± 1	93 ± 5	91 ±2	92 ± 3	98 ± 2	94 ± 1	88 ± 2	91 ± 5	93 ± 4
Sulfamethoxazole	93 ± 5	104 ± 7	92 ± 3	97 ± 5	95 ± 2	96 ± 1	91 ± 4	84 ± 1	85 ± 2	89 ± 6
p-Nitrophenol	95 ± 2	90 ± 4	102 ± 6	92 ± 3	94 ± 5	104 ± 4	105 ± 2	116 ± 4	112 ± 1	109 ± 3

TW: Tap water; SW: Surface water; $WWBO_3$: Wastewater before ozonation; $WWAO_3$: Wastewater after ozonation; WWFE: Wastewater final effluent

Table 2.8: Intra-day and inter-day precision for target compounds in all water matrices

Compound	Intra-day, RSD (%, n=7)					Inter-day, RSD (%, n=7)				
	TW	SW	$WWBO_3$	$WWAO_3$	WWFE	TW	SW	$WWBO_3$	$WWAO_3$	WWFE
1H-Benzotriazole	3.7	3.0	2.0	1.5	4.7	3.0	4.3	7.6	1.8	5.7
Bisphenol A	2.8	5.2	2.4	3.1	1.8	4.3	6.0	5.8	7.3	2.8
Catechol	1.9	2.4	4.3	1.8	3.6	2.7	3.4	6.3	5.1	8.5
p-Benzoquinone	4.1	3.2	5.2	2.5	1.0	5.1	5.7	8.1	4.2	2.9
c,c-Muconic acid	2.5	0.8	3.4	3.1	1.4	4.6	8.3	2.4	4.9	5.2
t,t-Muconic acid	3.2	5.0	2.8	4.6	3.8	6.2	6.7	5.6	3.3	7.9
Carbamazepine	0.9	3.5	1.9	2.7	0.9	3.8	4.1	2.9	5.7	8.3
Anthranilic acid	1.1	2.4	2.1	3.6	1.7	7.5	2.9	8.7	4.8	6.4
Glyoxylic acid	2.6	3.1	4.0	2.2	1.3	4.9	8.4	3.5	7.2	5.0
Oxamic acid	4.9	0.9	2.7	4.3	2.1	2.5	7.2	8.2	3.6	4.4
Ciprofloxacin	3.5	1.2	1.8	3.7	2.5	6.7	2.4	3.6	6.0	5.1
Diclofenac	2.2	4.6	2.5	4.1	1.8	3.4	4.7	5.8	3.2	6.8
2,6-Dichloroaniline	0.7	2.4	2.9	3.3	4.2	2.8	5.4	3.4	2.9	7.2
Metoprolol	4.2	3.9	2.2	1.7	3.0	5.3	3.6	4.1	7.8	2.2
Paracetamol	3.7	2.0	2.6	3.8	1.4	7.6	2.8	5.2	3.7	7.0
Oxalic acid	2.3	0.8	4.4	2.6	1.7	3.3	6.1	2.6	7.4	4.9
Oxaloacetic acid	1.8	2.8	5.3	1.9	3.3	4.2	4.0	4.8	2.9	6.3
Malic acid	4.1	4.6	2.9	2.4	0.9	2.9	10.3	7.3	4.6	6.9
Malonic acid	3.4	2.7	4.8	3.1	1.2	6.8	8.4	5.4	6.1	4.5
Maleic acid	0.9	1.5	3.7	4.9	4.0	4.0	3.7	2.1	5.9	8.3
Succinic acid	1.3	3.4	2.1	4.7	2.6	3.2	9.2	4.5	2.6	7.1
1,2,4-Benzenetriol	4.6	2.8	5.9	2.9	1.4	7.1	3.8	6.3	3.9	7.7
Hydroquinone	2.8	2.3	3.4	1.5	3.0	5.6	4.9	7.4	5.2	6.5
Sulfamethoxazole	5.0	1.1	2.5	4.0	3.8	4.8	8.0	3.0	7.2	5.4
p-Nitrophenol	1.7	3.9	5.6	2.7	4.2	3.5	2.9	6.2	3.7	4.1

TW: Tap water; SW: Surface water; WWBO3: Wastewater before ozonation; WWAO3: Wastewater after ozonation; WWFE: Wastewater final effluent

2.4.4 Environmental application

The optimized SPE-LC-ESI-MS/MS method was applied for two sampling campaigns conducted in 2014 and 2015 at the Ruhr river and a WWTP located in North-Western Germany.

As can be deduced from Fig. 2.2, all parent analytes were ubiquitous in WW and SW samples. The concentrations in WW were generally in the range of a few ng/L to several thousand ng/L depending on the treatment step and sampling period. In 2014, the levels of compounds in $WWBO_3$ ranged between 391 (bisphenol A) and 3020 (carbamazepine) ng/L, 143 (bisphenol A) and 995 (carbamazepine) ng/L in $WWAO_3$, and between 52 (bisphenol A) and 602 (carbamazepine) ng/L in WWFE. In 2015, the concentrations varied from 227 (bisphenol A) to 3410 (carbamazepine) ng/L in $WWBO_3$, from 94 (bisphenol A) to 1190 (carbamazepine) ng/L in $WWAO_3$, and from 43 (bisphenol A) to 652 (carbamazepine) ng/L in WWFE.

As a result, the highest concentrations of all precursor compounds in two campaigns were obtained in $WWBO_3$ samples followed by $WWAO_3$, and then WWFE. This showed the degradation efficiency during advanced WWT using ozonation followed by a biologically active step. On the other hand, the TPs were detected only at low ng/L levels if at all, i.e., at much smaller concentrations than the parent analytes. Some analytes like hydroquinone and malonic acid were not detected in any of 2014 WW samples, same as glyoxylic acid and oxaloacetic acid in 2015. It can be noticed that 2014 and 2015 $WWAO_3$ showed the highest concentration levels for TPs (if detected) comparing to the other WW samples.

Regarding SW, all parent compounds were detected and quantified in both 2014 and 2015 campaigns but with lower concentrations than in WW samples. The concentrations ranged from 24 ng/L for bisphenol A to 129 ng/L for carbamazepine in 2014, while from 23 ng/L for ciprofloxacin to 166 ng/L for carbamazepine in 2015. TPs if present were at low concentrations (<50 ng/L) at both sampling dates. 1,2,4-benzenetriol, p-benzoquinone, 2,6-dichloroaniline, glyoxylic acid, hydroquinone, malonic acid, oxalic acid, oxaloacetic acid and oxamic acid, were not found in 2014 water samples, whereas several TPs were not detected too in 2015 such as 2,6-dichloroaniline, glyoxylic acid, hydroquinone, malonic acid, c,c-muconic acid, t,t-muconic acid, oxaloacetic acid and oxamic acid. Succinic acid (30 ng/L) and malic acid (41 ng/L) were the predominant substances in 2014 and 2015 SW samples, respectively. The concentration values are presented in Table 5.10. TPs were observed to be at high concentrations in $WWAO_3$ comparing to other WW and SW samples, which as a result confirms the formation of these compounds during an ozonation step. For all TPs a subsequent reduction of their concentration was noticed in both

WWFE and SW samples. This demonstrates the transient nature of the investigated TPS, i.e., their rapid further degradation in biologically active systems.

All parent analytes were found in German SW and WW samples at concentrations varying between ng/L and µg/L as reported previously [38-41]. TPs were examined first-time in our study and no previous data were available for comparison.

Figure 2.2: Concentration levels of parent compounds and TPs in water matrices at two sampling campaigns conducted in 2014 and 2015

2.5 Conclusions

Ten different SPE sorbents were compared for the isolation of twenty five selected micropollutants and TPs in water. Several procedures for sample pH adjustment and elution solvents have been investigated. Oasis and Strata SPE families showed comparable recovery values for the examined analytes. The best performance was achieved by combining strong anion and cation exchangers (Oasis MAX and Oasis MCX or Strata-X-A and Strata-X-C) in a tandem mode without any need for pH adjustment. This configuration yielded quantitative recoveries (≥ 90%) for all tested analytes. The application of the optimized method to TW, SW and several WW samples demonstrated its robustness to enrich and isolate acidic, neutral and basic compounds. The developed method represents a general extraction protocol and may solve several sample preparation problems since so far no procedure to enrich different compound classes from complicated water matrices with high recovery rates was available. Nobody yet described the use of tandem cartridges of strong anion and cation exchange materials and reported high recoveries for compounds with various physicochemical properties such as we achieved in this study. In so doing, the improved SPE method aids in satisfying the environmental community's need for reliable sample preparation methods to extract a wide range of compound classes in aquatic environments; especially those TPs with unknown ecotoxicological impacts produced during WWT.

The study results showed also the developed LC-ESI-MS/MS method was robust and sensitive for simultaneous detection and quantification of target analytes in SW and WW samples collected during two sampling campaigns at the nanograms-per-liter concentration range. Trace analysis of TPs in real waters was done for the first time in our study. The proposed method showed good linearity, and intra- and inter-day precision. Moreover, various water matrices did not affect the analysis in the LC–MS/MS, which implies that clean extracts are obtained. This might allow in the future to use external calibration instead of standard addition for quantification. The concentration levels of parent analytes decreased after ozonation and further biological post-treatment, which confirms the removal efficiency of these advanced treatment steps. Concentration levels of the detected TPs were in the lower ng/L range in all water samples. The highest values of these compounds were observed in $WWAO_3$ samples which in most cases proved the formation of new oxidation products after ozonation step. Furthermore, the presence of TPs in environmental water samples indicates that the results of laboratory experiments for degradation of compounds with ozone may be transferred to real treatment plant conditions.

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3 Suspect screening of micropollutants and their transformation products in advanced wastewater treatment

3.1 Abstract

Transformation products (TPs) of organic micropollutants are still rarely considered in aquatic environments. Many of these compounds can potentially be formed in the environment after biological or chemical degradation and analytical standards are typically lacking, therefore knowledge on the prevalence in aquatic environments remains deficient.

In this study, the efficiency of a suspect screening strategy using solid phase extraction with broad enrichment efficiency, followed by liquid chromatography-electrospray ionization-quadrupole-time-of-flight-mass spectrometry (LC–ESI-Q-TOF-MS) without reference standards was systematically evaluated for assessing the potential exposure of different compound classes and their ozonated TPs in surface water and several wastewater samples collected at different steps of an advanced treatment processes including ozonation. An automated molecular-feature analysis based on a list of 245 previously reported compounds and their TPs was used. Thresholds for blank subtraction, mass accuracy (5 ppm tolerance), peak height (minimum 1000 counts) and isotopic pattern score (≥ 80%) were applied to filter the picked peaks. The results showed that the number of successfully detected compounds using the search criteria was 189. A decrease in concentration levels was observed for parent compounds in wastewater after ozonation and after biological treatment processes, while formation of tentative TPs after ozonation accompanied by degradation after biological treatment was noticed. Some of the detected compounds were also found in surface water. Moreover, a plausible reliability for compound prediction was obtained when using relative retention time information as comparison criteria. Overall, the screening approach was fast and successful and can be expanded to other compound classes and TPs where reference standards are not readily accessible.

3.2 Introduction

A large number of chemicals used in households and industry including pharmaceuticals and personal care products (PPCPs), biocides, pesticides and many industrial chemicals are released into aquatic environments due to their incomplete removal in wastewater treatment plants (WWTPs) [1, 2].The occurrence and fate of contaminants of emerging concern in the environment has been studied extensively. Yet, relatively little attention has been paid to their transformation products (TPs) [3]. TPs of emerging contaminants can be found in the environment or WWTPs as a result of biotic and abiotic processes acting on parent compounds such as oxidation [4], hydrolysis [5], photolysis [6] and microbial metabolism [7]. TPs can be of significant concern especially if they reveal a biological activity or resistance to biodegradation [8-10]. Some TPs are equally active or even more active than the parent compounds on aquatic ecosystems and/or on humans [11-13]. However, many of the TPs are still undiscovered, and there is only little known with regard to their further environmental fate [14]. Lately, an international expert workshop concluded that the risks assessment of environmental TPs of PPCPs is among the top twenty key issues that need to be tackled by the research community [15].

Recently, researchers have started to study the formation of TPs during ozonation, which is considered one of the most promising technologies for advanced wastewater treatment [16]. Identification of TPs mostly relies on mass spectrometry [17]. In addition to the direct reaction of ozone with many organic molecules, its decomposition is initially fast and produces hydroxyl radicals as secondary strong oxidant that nonselectively oxidizes nearly all organic compounds [18-20].

Undoubtedly, identification of TPs is a great challenge in environmental analysis. One of the reasons is the lack of analytical reference standards for most potential TPs. Furthermore, it is still obviously unknown to which extent results of laboratory degradation studies are representative of actual environmental conditions [21].

To overcome the limitations of unit resolution mass spectrometers, the recent emergence of modern high resolution mass spectrometry (HRMS) has opened new windows of opportunity for the analysis of a wide range of knowns and unknowns in complex samples including parent compounds, metabolites and TPs [22, 23]. Several recent publications demonstrated differences between low-resolution (LR) MS and HR capabilities and testified the growing importance of HRMS [24, 25].

Quadrupole-time-of-flight (Q-TOF) is among the mostly used analyzers which has shown to enable fast, sensitive, and reliable detection and identification of low molecular weight substances,

even in the absence of reference standards [26-29]. It allows recording full-scan chromatograms with high mass accuracy and resolution that make it possible to selectively search for given TPs based on their exact masses [30]. Three major approaches for post-measurement processing were detailed by Krauss et al. [22]; target analysis (with reference standards), suspect screening (with suspected compounds based on prior information but without reference standards), and finally non-target screening (neither prior information nor reference standards are available).

Particularly suspect screening methods based on LC-HRMS have gained popularity [31]. Its main aim is detecting as many compounds as possible present in the samples. Subsequently, chromatograms can be searched for peaks with specific masses (calculated from the molecular formulas) to identify suspected targets from a list of compounds compiled beforehand. Thus, although exact mass screening methods are computationally rapid and many masses can be screened in a given sample, the gathering of evidence and confirmation of the screened masses remains very time-consuming. The application of suspect screening suffers from the large efforts of manual data evaluation. Consequently, systematic strategies with automated approaches are required to filter the search and facilitate "relevant" peaks identification.

The objective of this study was to use an automated suspect screening approach based on solid phase extraction and LC-HRMS to detect potential organic contaminants and their ozonated TPs in actual environmental water samples. A list containing the accurate mass of each compound was thereby the only information required a priori. Our work focused also on giving an overview for examination of the suspects' behavior (i.e. degradation and/or formation) in the studied WWTP after applying different advanced treatment processes.

3.3 Experimental

3.3.1 Chemicals

Methanol, acetonitrile, and water were supplied by Fischer Scientific GmbH (Nidderau, Germany) and were either of HPLC grade or LC-MS grade. Acetone (analytical grade), ammonium hydroxide (30%), ethanol (absolute), ethyl acetate (analytical grade) and formic acid (98-100%) were purchased from Merck (Darmstadt, Germany).

3.3.2 Sample collection and pretreatment

A sampling campaign was conducted in February, 2015 to grab four water samples.
Municipal 24 h composite wastewater samples were collected from urban WWTP in Duisburg-Vierlinden, Germany. Three samples were taken at different stages of treatment: wastewater before ozonation, wastewater after ozonation and wastewater final effluent after biological treatment. This WWTP is designed for 30000 equivalent inhabitants and to treat up to 60000 m^3/day of wastewater.
Surface water samples were collected from the Ruhr river at Essen-Werden, Germany. Wastewater of nearly two million inhabitants is discharged into the Ruhr river that on the other hand is also used as raw water source to supply drinking water via bank filtration.
All samples were collected in pre-cleaned amber glass bottles, transported immediately to the lab and stored in the dark at 4 ^oC until analysis in order to minimize degradation. Particulate matter was filtered just before extraction through a glass microfiber filter (GF/F, 0.7 μm average pore size, 47 mm diameter, Merck, Darmstadt, Germany). Filtered–samples were left without pH adjustment.

3.3.3 Sample extraction

Oasis MAX (200 mg, 6 mL) and Oasis MCX (200 mg, 6 mL) cartridges from Waters (Eschborn, Germany) were first conditioned and equilibrated with 2 x 3 mL methanol and 2 x 3 mL Milli-Q water respectively. Then, the two cartridges were connected together in tandem in which Oasis MAX was the cartridge connected directly to the sample reservoir while Oasis MCX was the subsequent one. 1 L water samples (including Milli-Q water as a blank) were passed through the cartridges by vacuum suction (maximum of 65 kPa) via large volume adapters at a flow rate of ~ 15 mL/min. After the extraction, the cartridges were disconnected, dried under vacuum for 30 minutes, wrapped in aluminum foil, and stored at -20 ^oC until washing and elution steps which were done within 24 h. The Oasis MAX cartridge was washed with 2 mL water-ammonia solution (95:5, v/v) and then eluted with 6 mL methanol-ethyl acetate-formic acid (69:29:2, v/v). Oasis MCX cartridge was washed and eluted with 2 mL water-formic acid (98:2, v/v) and 6 mL methanol-ethyl acetate-ammonia solution (67.5:27.5:5, v/v), respectively. Afterwards, the gathered eluates from both cartridges were mixed in a tube and reduced in volume under vacuum. The remaining solvent was changed to water and the final volume was set to 1 mL before transfer to HPLC vial (exact volume was determined by weighting the vials)

3.3.4 Liquid chromatography-quadrupole-time-of-flight-mass spectrometry

Liquid chromatography-electrospray ionization-quadrupole-time-of-flight-mass spectrometry (LC–ESI-Q-TOF-MS) operated in positive and negative ionization mode was used for analysis. The chromatographic separation was performed using a HPLC system (consisting of vacuum degasser, autosampler, and binary pump) (Agilent 1290 Infinity Series, Agilent Technologies) equipped with ProntoSIL C18 analytical column of 250 mm × 4.0 mm and 5.0 μm particle size (Bischoff, Leonberg, Germany). Gradient LC elution was performed with 0.1% formic acid in water as mobile phase A and 0.1% formic acid in acetonitrile as mobile phase B. For the analysis in both positive and negative mode, the optimized chromatographic method held the initial mobile phase composition (90% A) constant for 10 min, followed by a decrease in composition A to 27.5% within 25 min, then to 0% in 10 min, kept there for 10 min, and finally up to 90% in 2 min. A 10-min post-run time back to the initial mobile phase composition was used after each analysis. The flow rate and injection volume were set in both modes to 0.5 mL min^{-1} and 20 μL, respectively.

The HPLC system was connected to an Agilent 6560 Series Ion Mobility Q-TOF-MS (Agilent Technologies, Waldbronn, Germany). The instrument was operated in the 4 GHz HR mode. Ions are generated using an electrospray ion source with Agilent Jet Stream Technology. Full-scan HRMS data were recorded within a mass-to-charge (*m/z*) range of 45-1700 for each sample. Parameters for the Agilent Jet Stream Technology are the super-heated nitrogen sheath gas temperature (325 °C) and flow rate (12 L/min). Electrospray conditions were the following: capillary, 5000 V; nebulizer, 20 psi; drying gas, 5 L/min; gas temperature, 200 °C; skimmer 1 voltage, -30 V; octapoleRFPeak, 750 V; fragmentor (in-source CID fragmentation), 275 V. The mass axis was calibrated using the mixtures provided by the manufacturer over specific *m*/*z* values. A sprayer with a reference solution was used as continuous calibration in ESI+ mode using the following reference masses: 121.050873 and 922.009798 *m*/*z*. With ESI- mode, reference masses were 119.036320 and 966.000725 *m*/*z*. For this work, the Ion Mobility Q-TOF-MS instrument was used as a Q-TOF system working in the MS mode for detection and identification of suspect compounds. The full-scan data recorder was processed with Agilent MassHunter Workstation Software (version B.06.00).

3.3.5 Optimization of the suspect screening

The theoretical monoisotopic exact mass, which was the only information a priori, was calculated for each compound based on the molecular formula using the Isotope Distribution Calculator tool of the Agilent software. The gathered data including compound name and exact mass were put in an Excel spreadsheet and saved into csv format. The created csv Excel file was employed as a database to be searched by the instrument software of the LC-Q-TOF-MS system.

As ESI generally produces molecular ions $[M+H]^+$ or $[M-H]^-$ [32], suspect screening was performed using these masses. Other adducts (e.g., Na^+, K^+, NH_4^+, HCO_2^-, and $H_3C_2O_2^-$) were not included for simplicity. Due to the difficulty of predicting ionization behavior, all suspected substances were screened in positive and negative ionization mode in order to avoid missing compounds at the beginning of the workflow. Owing to the complexity of water samples, some filters were applied to reduce the total number of compounds extracted. Search criteria included an accurate mass tolerance of 5 ppm and an absolute abundance higher or equal to the height of 1000 counts. Peaks from a blank sample were also subtracted for each detected compound.

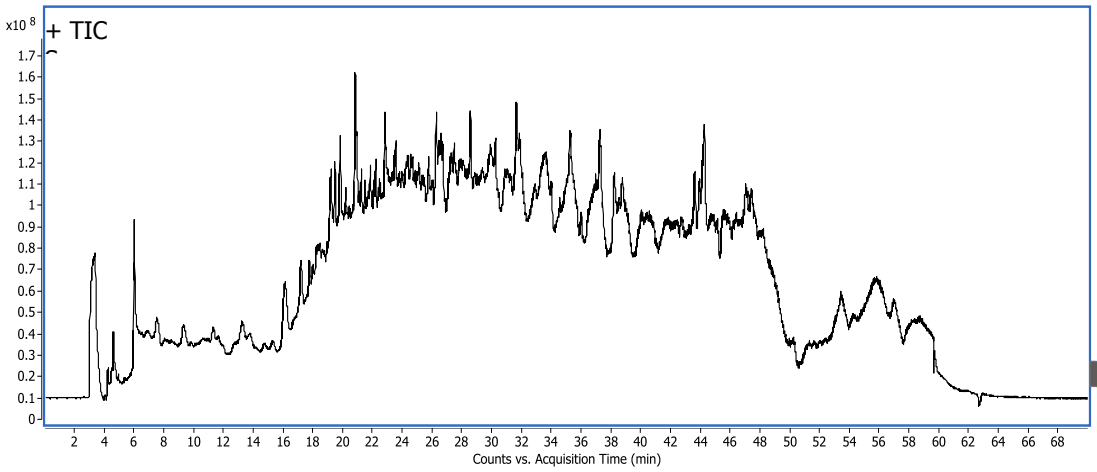
Taking into account the accurate mass, the software provides a score value, which is used to evaluate database search results. The score is reported on a scale of 0 to 100. When a formula is available as a database entry or target compound, a combined score is calculated which is based on mass, isotope abundance and isotope spacing. The overall score for a formula is calculated as a weighted average of individual probabilities. The automatic filter criteria for the compound quality score was set to be $\geq 80\%$

3.4 Results and discussion

A suspect list of parent compounds and tentative TPs produced during ozonation in laboratory experiments was compiled from literature and is summarized in Table 5.11.

The automated screening method of the database is called a molecular-feature extraction algorithm software (Qualitative Mass Hunter). This software examines the whole chromatogram at once in order to search and group all ions which represent real compounds. The search software then compares the ions found with the specified adducts in the compound database and then creates a compound list for peaks which might represent real molecules.

Fig. 3.1 shows an example of a total ion chromatogram of a wastewater sample after ozonation with an excerpt of the database result using the automatic screening method.



Compound Name	Mass (DB)	Formula (DB)	RT	m/z	Diff (ppm)	Score
Trimethoprim (TMP)	290.1379	C14H18N4O3	40.05	291.1455	-0.69	99.33
TMP-TP1	294.1328	C13H18N4O4	21.83	295.1412	2.03	89.23
Ketoprofen (KPR)	254.0943	C16H14O3	32.35	255.1026	1.96	97.07
KPR-TP2	226.0994	C15H14O2	30.30	227.1078	2.64	87.78
Clarithromycin (CMC)	747.4769	C38H69NO13	25.79	748.4842	-0.67	98.04
CMC-TP1	763.4718	C38H69NO14	23.08	764.4789	-0.92	96.92

Figure 3.1: Total ion chromatogram and a short list database obtained by LC-Q-TOF-MS screening of a wastewater sample after ozonation

In order to isolate and enrich the analytes from water samples, a solid phase extraction method was previously developed using different compound classes and their ozonated TPs as target analytes.

The concept of confirmation by accurate mass has been addressed by many researchers in the field of water analysis (e.g. [33-35]).

The reliability of the screening method depends on the ruggedness of the TOF instrument to provide consistently accurate mass measurements within a fixed mass error tolerance. Typically, the measurement of accurate masses within 5 ppm is widely accepted for the verification of the elemental composition [36]. The Q-TOF system used in this work has demonstrated mass accuracy values of < 4 ppm in all cases, regardless of the matrix or the concentration level (see Table 5.12). Based on the high mass accuracy unknown compounds can be assigned with a sum formula and it is often possible to differentiate between compounds with the same nominal mass but different elemental composition which will have different exact masses. With low-resolution mass spectrometry it is not possible to distinguish isobaric species. For example, ATL-TP2 and KPR-TP4 have the same nominal masses of 224.26 and 224.25, respectively, but differ in their sum formula and the instrument was able to discriminate between them according to their exact mass.

In addition to the use of accurate mass, database searching with isotope pattern recognition will enhance the performance of the method and provide extra information to the findings based on the isotopic signals. This is particularly useful for confirmatory purposes on those compounds containing chlorine, bromine, sulfur, etc. The application of this filter allowed a reduction in the number of proposed elemental compositions that would fit for a certain mass window. Consequently, isotopic score values ≥ 80% were obtained for all suspects.

The score value is calculated by the software considering not only the accurate mass but also the isotopic distribution. As an example to illustrate the usefulness of this parameter to match the findings, the mass, isotope abundance and isotope spacing for diclofenac TP (DFC-TP1), which has a formula of $C_{14}H_{11}Cl_2NO_3$, are shown in Fig. 3.2. Anyway, the score value in this case was 100.

Note that the accurate mass defect when going from the X peak to the X + 2 and the X + 4 peak was 1.997 mass unit, which is a result of the presence of the ^{37}Cl isotope, and is accounted for by the difference in mass between ^{35}Cl and ^{37}Cl. Also note that the abundances of the X + 2 and X + 4 isotopic patterns match a compound containing two Cl atoms. In addition, the X + 1 isotope shows a positive mass defect of 1.0033 mass unit for ^{13}C.

Examining data in Table 5.13, which show the peak areas for compounds in different water matrices, it was noted that all 34 parent compounds were detected in all four samples except imazalil, which was not presented in both wastewater effluent and surface water samples. Acyclovir was detected in wastewater samples but not found in surface water. The number of integrated TPs peaks was 155 compared to a total TPs number of 211. Roughly, 100% of the detected TPs were present in wastewater after ozonation, while 9%, 52% and 34% were determined in wastewater before ozonation, wastewater final effluent and surface water, respectively.

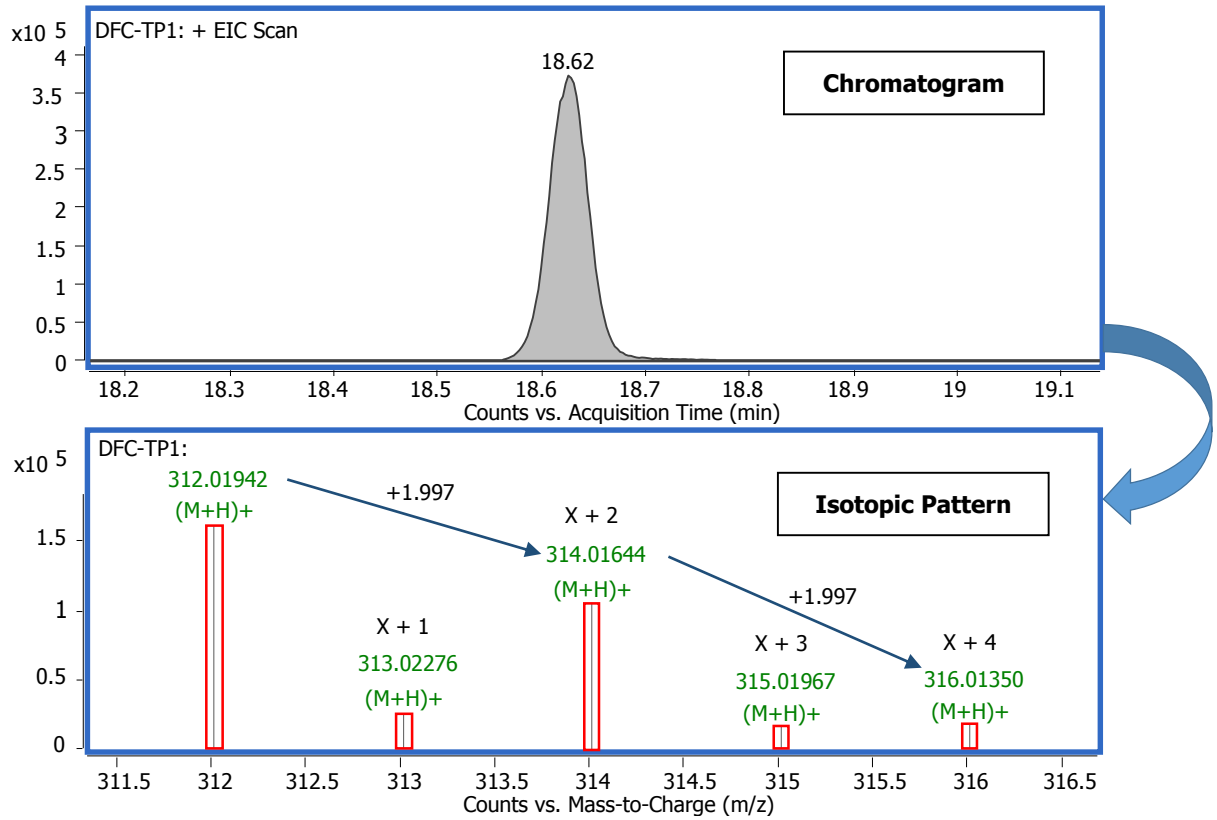


Figure 3.2: Example for the detected substance (diclofenac transformation product DFC-TP1) in the suspect screening showing a chromatogram peak and measured spectra

The suspect compounds were present in environmental water samples at variable concentration values deduced from the largely varying peak areas. Relative peak area (RPA) was calculated for each compound. Fig. 3.3 summarizes the statistical data of the presumed compounds in box-whisker plots. For parent compounds, the highest peak areas were recorded for compounds in wastewater before ozonation, so other values were calculated relative to it. An obvious decrease in RPAs was observed for all compounds after ozonation and further biological post-treatment. The results indicate that these compounds have been discharged into WWTP at high concentrations and degraded partially or completely after advanced treatment and then released into surface water but at low levels.

Regarding TPs, highest PAs were obtained for compounds in wastewater after ozonation and represented a basis for comparison where RPAs were calculated in other water samples. Almost zero RPAs were estimated for the suspects in wastewater before ozonation while decreasing in the values after adding bio-filters (final effluent) was perceived. Due to the elevated concentrations of the TPs in wastewater after ozonation, most of chemical formulas might belong to compounds formed after applying ozonation as a treatment process. However, some of these compounds were also detected in surface water as a result of formation and emission via WWTP.

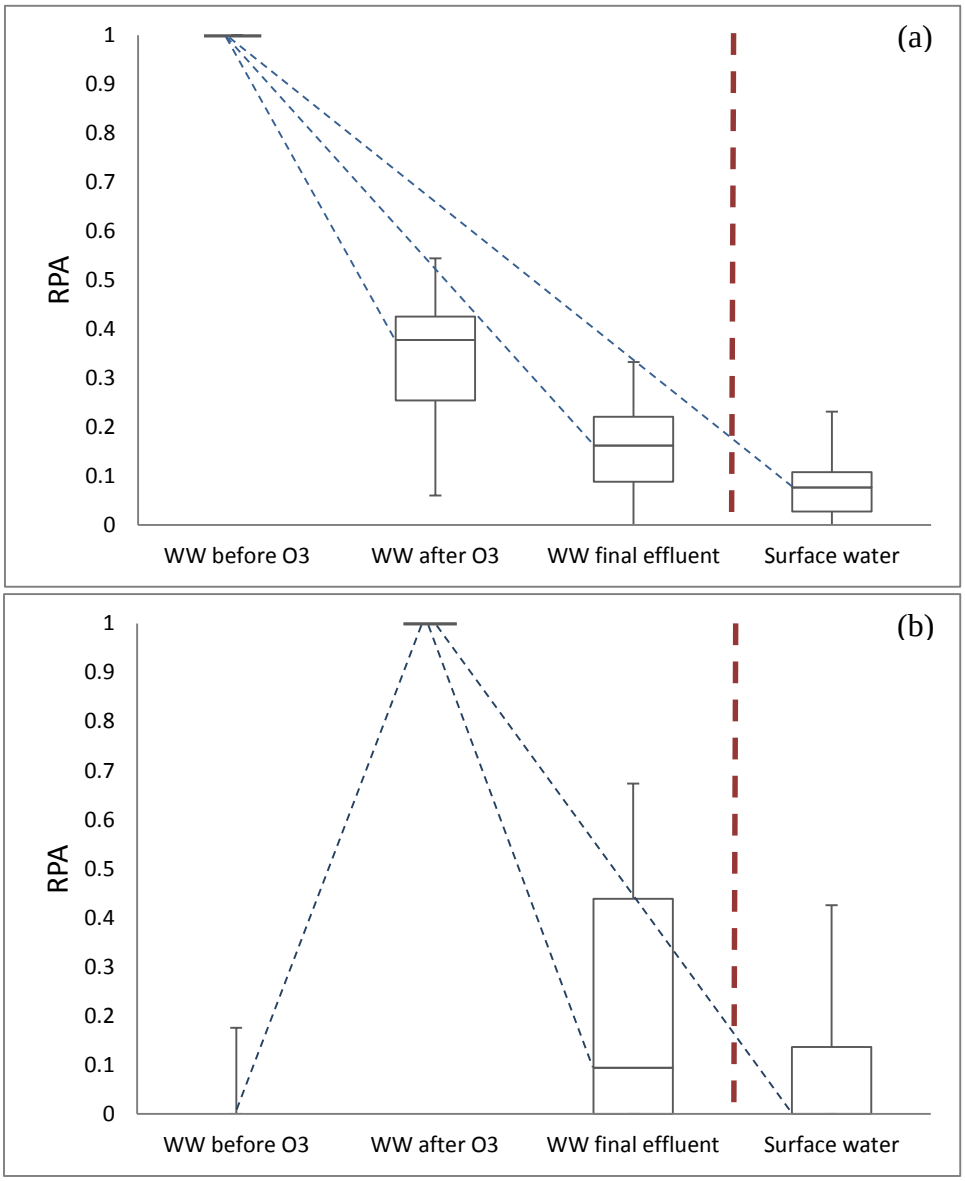


Figure 3.3: Example for the detected substance (diclofenac transformation product DFC-TP1) in the suspect screening showing a chromatogram peak and measured spectra

WW before O_3: wastewater before ozonation; WW after O_3: wastewater after ozonation

To use a retention time (RT) of the analyte as an additional criterion to explain our findings, relative retention times (RRT) of the TPs need to be evaluated. They are calculated by dividing the RT of each TP to the RT of its parent compound and then compare the results with the values obtained from previous studies in case of availability as listed in Table 5.11. Since C18 is the HPLC stationary phase used in all cases, we can expect a similar retention behavior for TPs relative to their parent compounds. Fig. 3.4 shows a plot of RRTs obtained from our work and previous studies. As can be seen, RRTs matched very well. As an example, bisphenol A (BPA) TPs 1-3 were released before BPA (RRT <1) as in literature and in the same order where BPA-TP1 eluted

first, then BPA-TP2 and finally BPA-TP3. Another example is Roxithromycin (ROX) where RRTs were <1 for ROX-TP1 and ROX-TP2 while RRTs >1 were obtained for ROX-TP3, 4, 5 in the same elution order for all. Remaining deviations can be explained due to the use of kind and percentage of organic modifiers chosen in each study.

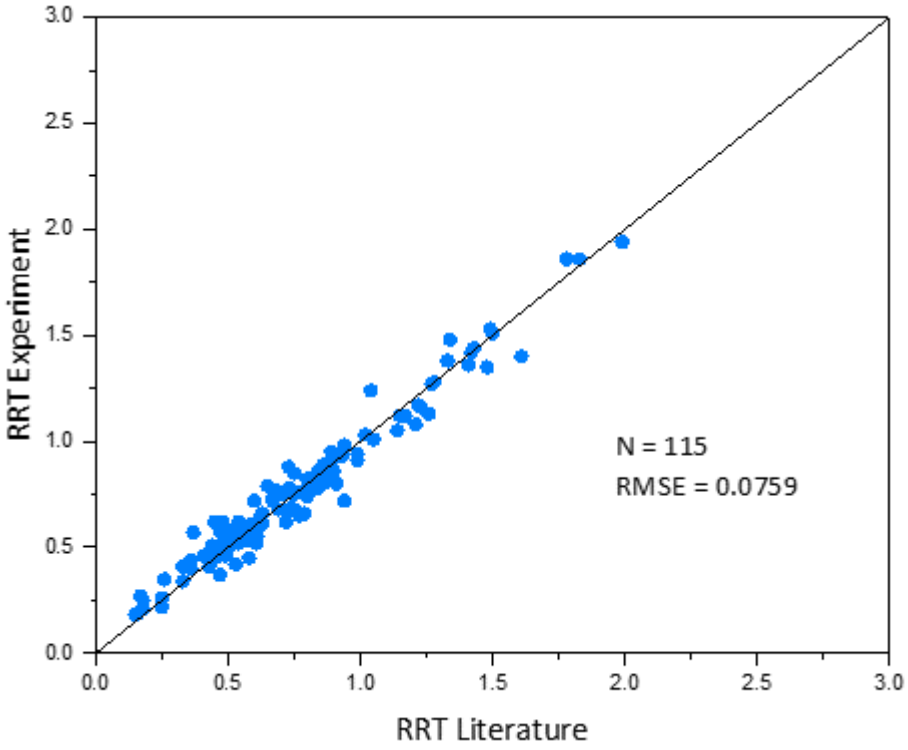


Figure 3.4: Relative retention time (RRT) comparison for TPs between experimental results and literature values. Solid line represents the 1:1 fit, N: number of data points, RMSE: root mean squared error

3.5 Conclusions

A combination of nonselective extraction together with selective and sensitive detection by HRMS allowed the establishment of a suspect screening approach without reference standards, covering emerging contaminants and their tentative TPs in surface water and wastewater samples taken after different treatment processes. The Q-TOF molecular-feature extraction algorithm to screen for compounds using only exact mass as a priori information was proven to be fast and effective when accompanied by automatic filters such as mass tolerance, peak intensity, and isotope pattern recognition. Mass accuracy < 5 ppm and isotopic score ≥ 80% were obtained for all detected suspects regardless of the complexity of water samples. A decrease in peak areas, which corresponds to decrease in concentrations, were observed for all parent compounds in wastewater after ozonation, followed by wastewater final effluent, and then surface water. This is an indication that the studied WWTP is working well to degrade these compounds.

In contrast, the detected TPs were found to be at higher relative concentration levels in wastewater after ozonation, however, a decrease or disappearance was observed in wastewater final effluent and surface water. Almost no peaks were found in wastewater before ozonation. This demonstrates the formation of these compounds during treatment with ozone as well as subsequent reduction of their concentration in biologically active systems. Furthermore, the results also showed a plausible matching between RRTs in our study and values obtained from literatures.

Commonly, laboratory studies offer the advantage of proposing TPs under well-defined conditions with appropriate control that facilitates the establishment of differences in the samples that contain the compounds. However, the identification of these compounds in the environment is still rare. Therefore, the sample preparation and analytical approach introduced in this study in addition to the collected suspect list will be very useful to further study and confirm our results using MS/MS and/or other methodologies.

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4 General conclusions and outlook

The results obtained in this thesis clearly demonstrate that transformation products (TPs) produced after ozonation are of potential environmental concern. A proper sample preparation method that would allow isolation of such compounds and others from aquatic environments is missing. Solid phase extraction (SPE) has been widely used in many applications and proven to be an effective technique especially in environmental water area. Development of a SPE method is a challenging task due to the fact that organic substances vary in their characteristics and therefore several parameters like the selection of sorbent, adjustment the sample pH, selection of organic solvents for washing and elution steps, and others are needed to be well optimized. The method will not only enhance the analytical analysis but also will help the biologists to examine the potential toxicological effects that might harm the environmental systems; since they mostly use random SPE sorbents for the enrichment of water samples without testing their selectivity in advance.

During the development of a SPE method, it was noticed that leaving water samples without pH adjustment was the simplest and more appropriate option since no significant effect was observed. Also, the tested materials from both Phenomenex and Waters manufacturers gave comparable results. The final developed procedure allowed the enrichment of hydrophilic-lipophilic-anionic-cationic compounds. The improved SPE procedure was successfully transferred to real water samples (tap water, surface water and several wastewaters), and recoveries between 90 and 110% were obtained. This is the first time in research area to describe the use of strong anion and cation exchange sorbents in tandem in order to extract a wide variety of organic compounds from different water bodies at high recovery rates. Studying the effect of other factors on recovery like water sample volume, sample loading flow rate, and cartridges storage time (after enrichment and before elution), will be significant for future investigations.

Robust and sensitive analytical method for the analysis of micropollutants and TPs was developed. This allowed, for the first time, the detection of target TPs in the aquatic environment. However, no significant unwanted effects were observed when utilizing mass spectrometry (MS) as a detection technique even in the presence of complex matrices. This was due to the high efficiency of SPE procedure to reduce or eliminate the concentration of interfering substances which influence the ionization yields. It is therefore, recommended to those developing methods for trace enrichment of organic micropollutants in complex matrices that selective sample clean-up will be

sufficient in order to achieve the desired levels of sensitivity with no effects on analyte recovery. Adding a surrogate standard to samples prior to extraction would be useful in the future for quantification purposes. A notable conclusion from the research undertaken in this study is that a general and reliable SPE protocol was presented for both enrichment and clean-up purposes, therefore it is recommended to be used in the field of environmental water analysis.

It is shown that all parent compounds were detected in wastewater samples after each treatment step in two sampling campaigns. A decrease in their concentrations was observed after ozonation and further biological post-treatment. The results obtained highlight that the degradation of parent compounds in advanced wastewater treatment via ozonation is in general not leading to a complete mineralization but rather results in the formation of more polar TPs, and biological treatment process in a final polishing step was capable to degrade them partially or completely. Furthermore, the presence of all parent compounds and some TPs in the Ruhr river is attributed to their incomplete removal in WWTP even after biological treatment.

Studying the occurrence of TPs in environmental water matrices is highly challenging. Many laboratory studies focused on the ozonation of organic compounds and proposing molecular structures for the newly formed TPs but only few of them tracked these compounds in real water samples. The main reason was the lack of reference standards. HRMS instruments, such as Q-TOF-MS, opened up a new horizon to overcome the limitation of unavailable authentic standards by screening compounds depending only on their accurate masses. Therefore, molecular formulas and exact masses for a wide spectrum of organic micropollutants and their ozonated TPs were compiled and search criteria defined in order to reduce the number of hits. The results showed that all parent compounds were at higher concentrations in wastewater before ozonation, followed by a decrease after ozonation and after biological treatment. Also, TPs were found at high levels in wastewater after ozonation, which means these compounds were formed as a result of using ozone. Some of the suspects were also detected in surface water but at lower concentrations than in wastewater samples. Furthermore, a good matching was observed between the obtained values of relative retention times and the reported ones from previous studies. This is crucial to assess whether TPs can be more or less polar than parent compounds as well as among themselves.

The results of suspect screening gave a general picture to the possible formation of TPs in WWTP and the discharge to receiving waters. Therefore, this subject must be studied widely because these compounds might complicate the situation when they are of more concern than parent compounds even if they present at low concentration levels. A future challenge would be to test the efficiency

of different treatment processes for their ability to remove such compounds in order to be implemented after ozonation in addition to biological treatment.

The inclusion of more micropollutants into analysis list using the developed SPE method should be addressed in the future. The behavior of these compounds during wastewater treatment processes requires investigation starting from influent to the final effluent using high frequency flow dependent sampling. This should also be expanded by studying several WWTPs and other environmental matrices like surface and ground waters. As this research focused on ozonated TPs, it will be necessary to study their presence in different WWTPs running ozonation by grabbing more samples and making comparisons during the same and different years. Another important thing is to expand the research by including another abiotic and biotic TPs and tracking them during wastewater treatment as well as in receiving waters by means of target or suspect screening depending on the availability of relevant standards. A future challenge would be to check the toxicological effects of compounds in the extracts, obtained after applying the developed SPE method in different environmental waters, using different bioassays especially in the presence of TPs where little information have been reported. This is of paramount importance to determine whether there is any danger to human health or unacceptable damage to the natural environment. From designed mixture toxicity studies, it is noticed that even if single chemicals are present below concentrations that cause a visible effect, they may contribute to the mixture effect [1, 2]. Chemical analysis of a limited suite of compounds does not allow assessment of the potential biological adverse effects of water sample as the cumulative effect of the mixture of chemicals that may be present cannot be easily integrated [3-5]. Bioassays have been utilized as complementary monitoring tools for the assessment of possible biological effects of chemicals that are present in a particular water sample [6, 7]. These bioanalytical tools are designed to quantify non-specific toxicity or particular toxic modes of action (e.g. estrogenicity, genotoxicity, phytotoxicity) induced by a sample on a biological organism or a biological process [3]. Moreover, studying the possibility of packing the two SPE sorbents in one cartridge and transferring the procedure to a fully automated SPE-LC-MS/MS system would save additional work and time. Further development on analytical methods seems to be necessary, using more sensitive UPLC-MS/MS systems to analyze the compounds of interest at less time and lower detection limits. Multidimensional LC separation is also an option to be considered especially in suspect and non-target screening of substances due to its advantage to provide sufficient resolving power for the separation of components in complex matrices [8-11]. In addition to LC, it is also necessary to take GC into consideration by developing a method for analysis of volatile compounds present in

the extracts. Another field for future research is to quantify and compare the matrix effects from large volume injection method and the improved SPE procedure for the analysis of contaminants in different water matrices using ESI and other ion sources. A further confirmation to the suspect screening findings is necessary by using MS^n fragmentation and/or NMR as a tool for unequivocal identification. The main drawback of NMR is the poor sensitivity compared to MS [12, 13], so it is more efficient to use SPE and NMR linked to MS [14].

4.1 References

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5 Supplementary

5.1 General introduction

No supplements

5.2 Tandem anion and cation exchange solid phase extraction for the enrichment of micropollutants and transformation products from ozonation

Table 5.1: Structures of compounds with pKa values

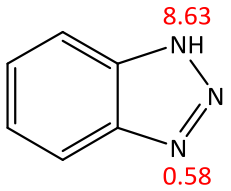
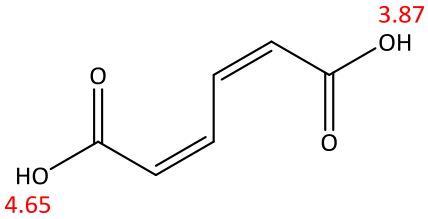
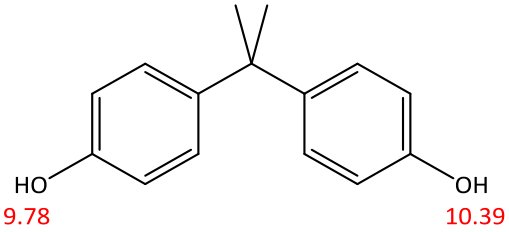
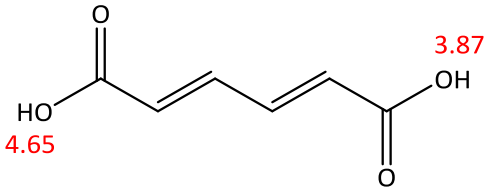
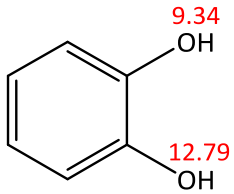
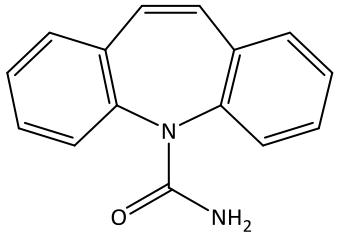
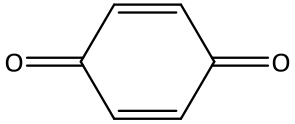
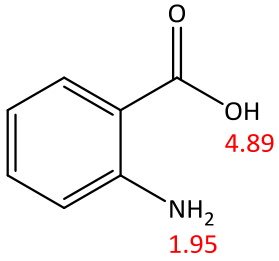
Compound	Chemical structure	Compound	Chemical structure
1H-Benzotriazole		c,c-Muconic acid	
Bisphenol A		t,t-Muconic acid	
Catechol		Carbamazepine	
p-Benzoquinone		Anthranilic acid	

Table 5.1: Structures of compounds with pKa values (continued)

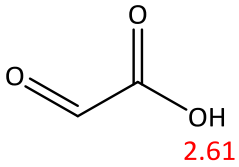
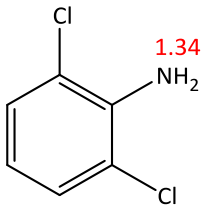
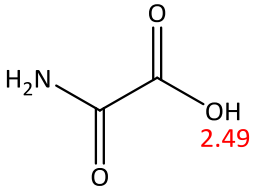
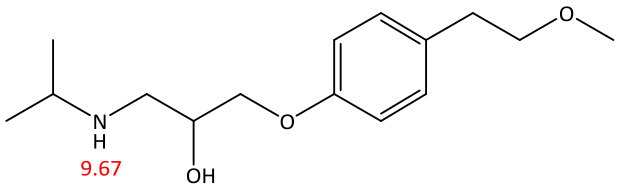
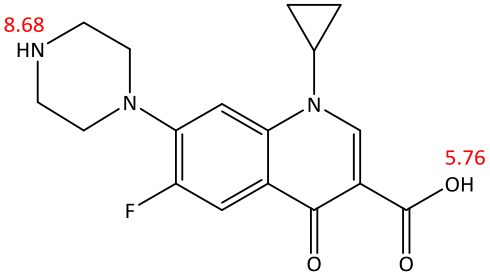
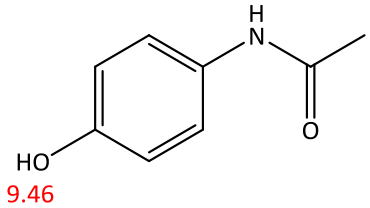
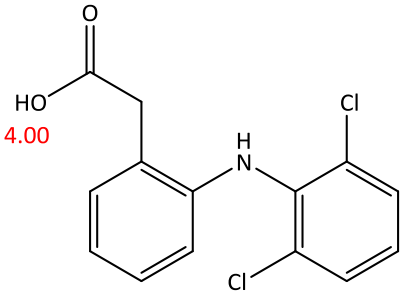
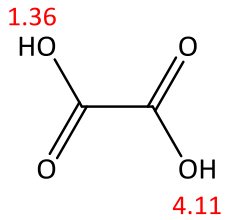
Compound	Chemical structure	Compound	Chemical structure
Glyoxylic acid		2,6-Dichloroaniline	
Oxamic acid		Metoprolol	
Ciprofloxacin		Paracetamol	
Diclofenac		Oxalic acid	

Table 5.1: Structures of compounds with pKa values (continued)

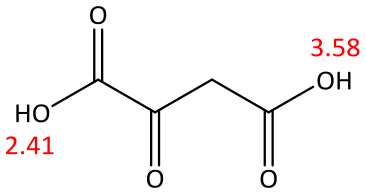
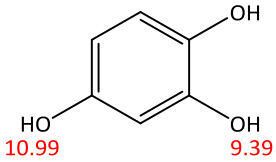
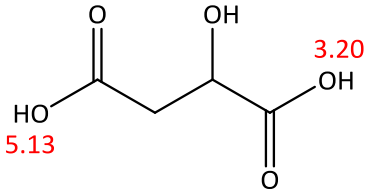
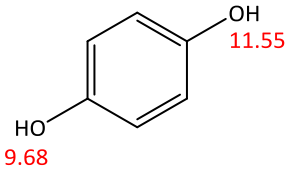
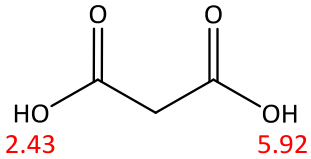
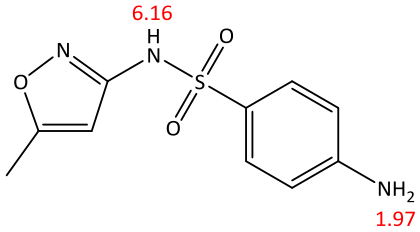
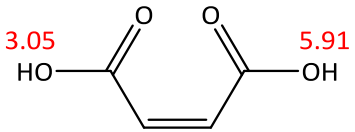
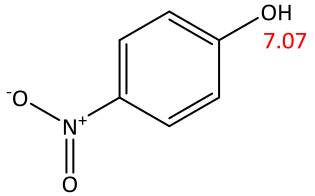
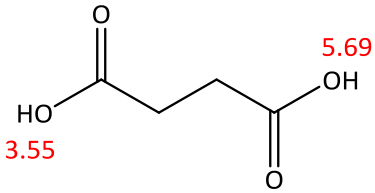
Compound	Chemical structure	Compound	Chemical structure
Oxaloacetic acid		1,2,4-Benzenetriol	
Malic acid		Hydroquinone	
Malonic acid		Sulfamethoxazole	
Maleic acid		p-Nitrophenol	
Succinic acid			

Table 5.2: Recoveries (%) and RSD (n=3) for different SPE materials obtained by eluting of compounds with ethyl acetate

Compound	Oasis HLB [1]	Oasis MAX [2]	Oasis MCX [3]	Oasis WAX [3]	Oasis WCX [2]	Strata-X [1]	Strata-X-A [2]	Strata-X-C [3]	Strata-X-AW [3]	Strata-X-CW [2]
1H-Benzotriazole	60 ± 4	54 ± 3	48 ± 5	43 ± 2	40 ± 1	55 ± 2	50 ± 3	46 ± 4	40 ± 3	37 ± 4
Bisphenol A	62 ± 1	57 ± 5	51 ± 2	40 ± 4	36 ± 3	64 ± 5	59 ± 1	50 ± 5	42 ± 1	39 ± 3
Catechol	56 ± 3	50 ± 2	45 ± 4	37 ± 1	32 ± 5	60 ± 1	55 ± 5	41 ± 2	39 ± 4	36 ± 1
p-Benzoquinone	59 ± 4	54 ± 1	50 ± 2	46 ± 3	44 ± 4	56 ± 4	50 ± 2	47 ± 4	42 ± 2	40 ± 5
c,c-Muconic acid	53 ± 3	65 ± 3	51 ± 5	40 ± 4	31 ± 2	51 ± 2	61 ± 3	50 ± 2	41 ± 5	35 ± 4
t,t-Muconic acid	51 ± 2	62 ± 4	47 ± 3	43 ± 3	38 ± 1	50 ± 3	65 ± 5	52 ± 2	46 ± 1	31 ± 3
Carbamazepine	60 ± 5	55 ± 2	52 ± 4	38 ± 1	35 ± 5	56 ± 1	52 ± 1	48 ± 5	33 ± 3	30 ± 2
Anthranilic acid	55 ± 1	60 ± 3	45 ± 2	40 ± 3	37 ± 4	54 ± 4	64 ± 4	50 ± 3	44 ± 2	38 ± 4
Glyoxylic acid	50 ± 3	63 ± 1	48 ± 3	43 ± 5	40 ± 1	46 ± 2	58 ± 2	42 ± 1	37 ± 3	33 ± 5
Oxamic acid	52 ± 2	61 ± 3	50 ± 2	41 ± 4	38 ± 2	55 ± 5	60 ± 1	48 ± 5	38 ± 2	32 ± 1
Ciprofloxacin	64 ± 3	68 ± 2	60 ± 4	45 ± 1	41 ± 2	60 ± 4	66 ± 5	57 ± 2	42 ± 4	30 ± 3
Diclofenac	60 ± 5	65 ± 4	57 ± 2	42 ± 3	39 ± 5	52 ± 1	69 ± 2	61 ± 4	46 ± 1	41 ± 4
2,6-Dichloroaniline	58 ± 1	52 ± 3	46 ± 3	37 ± 5	33 ± 2	54 ± 3	48 ± 4	42 ± 2	32 ± 3	30 ± 5
Metoprolol	54 ± 3	59 ± 5	58 ± 1	35 ± 4	36 ± 3	50 ± 2	54 ± 2	60 ± 3	38 ± 2	36 ± 2
Paracetamol	61 ± 4	55 ± 1	52 ± 2	45 ± 3	40 ± 5	64 ± 2	58 ± 3	55 ± 5	41 ± 4	39 ± 3
Oxalic acid	47 ± 2	61 ± 4	43 ± 5	40 ± 1	36 ± 2	49 ± 4	65 ± 1	47 ± 2	44 ± 5	40 ± 1
Oxaloacetic acid	52 ± 5	57 ± 2	47 ± 3	44 ± 2	34 ± 4	48 ± 1	54 ± 5	42 ± 1	40 ± 3	36 ± 3
Malic acid	45 ± 3	55 ± 4	42 ± 2	36 ± 5	33 ± 3	42 ± 5	51 ± 3	38 ± 4	35 ± 2	30 ± 5
Malonic acid	51 ± 1	62 ± 3	46 ± 2	42 ± 4	37 ± 1	55 ± 2	62 ± 1	50 ± 5	46 ± 1	41 ± 3
Maleic acid	54 ± 3	66 ± 1	50 ± 3	45 ± 2	38 ± 5	50 ± 4	61 ± 2	47 ± 1	40 ± 4	34 ± 2
Succinic acid	62 ± 2	65 ± 3	57 ± 1	40 ± 4	36 ± 2	60 ± 1	68 ± 4	54 ± 3	47 ± 2	40 ± 4
1,2,4-Benzenetriol	58 ± 5	52 ± 2	44 ± 4	38 ± 1	34 ± 3	54 ± 3	50 ± 2	40 ± 2	38 ± 1	31 ± 5
Hydroquinone	60 ± 3	54 ± 5	51 ± 2	35 ± 3	31 ± 1	64 ± 2	56 ± 3	54 ± 5	36 ± 4	33 ± 2
Sulfamethoxazole	53 ± 4	61 ± 2	49 ± 4	42 ± 5	36 ± 3	57 ± 5	65 ± 3	53 ± 1	40 ± 2	36 ± 1
p-Nitrophenol	61 ± 2	64 ± 3	57 ± 1	38 ± 3	40 ± 5	58 ± 4	61 ± 2	53 ± 3	35 ± 2	31 ± 4

[1] 100% ethyl acetate; [2] ethyl acetate-formic acid (98:2, v/v); [3] ethyl acetate-ammonia solution (95:5, v/v)

Table 5.3: Recoveries (%) and RSD (n=3) for different SPE materials obtained by eluting of compounds with methanol

Compound	Oasis HLB [1]	Oasis MAX [2]	Oasis MCX [3]	Oasis WAX [3]	Oasis WCX [2]	Strata-X [1]	Strata-X-A [2]	Strata-X-C [3]	Strata-X-AW [3]	Strata-X-CW [2]
1H-Benzotriazole	65 ± 3	68 ± 1	65 ± 2	51 ± 1	47 ± 5	67 ± 1	66 ± 4	62 ± 3	51 ± 5	45 ± 2
Bisphenol A	66 ± 2	69 ± 5	62 ± 1	53 ± 4	40 ± 3	70 ± 4	67 ± 2	66 ± 5	50 ± 2	42 ± 1
Catechol	62 ± 3	66 ± 2	63 ± 4	51 ± 2	50 ± 1	66 ± 2	62 ± 1	60 ± 2	47 ± 4	45 ± 3
p-Benzoquinone	66 ± 2	70 ± 4	65 ± 1	50 ± 3	46 ± 5	71 ± 3	70 ± 3	66 ± 4	53 ± 1	48 ± 2
c,c-Muconic acid	60 ± 5	71 ± 3	62 ± 5	45 ± 1	37 ± 4	57 ± 4	67 ± 5	58 ± 2	47 ± 3	40 ± 5
t,t-Muconic acid	55 ± 2	72 ± 5	60 ± 1	50 ± 4	40 ± 5	55 ± 2	72 ± 3	55 ± 3	52 ± 1	37 ± 3
Carbamazepine	67 ± 1	64 ± 3	61 ± 2	42 ± 3	38 ± 2	69 ± 5	68 ± 2	63 ± 1	39 ± 4	35 ± 1
Anthranilic acid	63 ± 3	72 ± 4	58 ± 3	47 ± 5	40 ± 4	60 ± 2	67 ± 5	53 ± 2	50 ± 2	42 ± 3
Glyoxylic acid	54 ± 5	76 ± 2	56 ± 3	45 ± 4	41 ± 1	55 ± 3	70 ± 1	60 ± 5	42 ± 3	38 ± 5
Oxamic acid	61 ± 2	70 ± 1	60 ± 4	46 ± 2	43 ± 2	57 ± 5	66 ± 2	52 ± 3	41 ± 5	37 ± 4
Ciprofloxacin	68 ± 1	74 ± 4	71 ± 2	50 ± 1	43 ± 3	65 ± 4	70 ± 2	63 ± 1	45 ± 3	38 ± 2
Diclofenac	63 ± 5	68 ± 3	65 ± 5	49 ± 2	44 ± 4	58 ± 2	74 ± 4	69 ± 3	52 ± 4	45 ± 1
2,6-Dichloroaniline	68 ± 4	65 ± 2	64 ± 3	46 ± 5	41 ± 2	70 ± 1	65 ± 3	60 ± 5	42 ± 1	38 ± 3
Metoprolol	62 ± 3	67 ± 5	60 ± 4	46 ± 3	40 ± 1	61 ± 4	64 ± 1	72 ± 2	44 ± 2	41 ± 4
Paracetamol	68 ± 5	64 ± 2	66 ± 1	47 ± 4	45 ± 2	71 ± 2	64 ± 3	60 ± 2	45 ± 5	42 ± 3
Oxalic acid	56 ± 2	70 ± 5	54 ± 2	50 ± 1	47 ± 5	58 ± 3	72 ± 2	52 ± 5	48 ± 3	43 ± 1
Oxaloacetic acid	60 ± 4	70 ± 3	63 ± 5	48 ± 4	43 ± 1	60 ± 2	68 ± 5	57 ± 4	44 ± 1	40 ± 5
Malic acid	52 ± 3	69 ± 1	56 ± 2	51 ± 3	45 ± 2	61 ± 5	71 ± 2	61 ± 1	47 ± 2	43 ± 3
Malonic acid	60 ± 2	72 ± 4	63 ± 1	45 ± 4	39 ± 5	57 ± 1	66 ± 3	56 ± 2	50 ± 1	43 ± 2
Maleic acid	57 ± 4	71 ± 3	60 ± 4	51 ± 1	42 ± 3	58 ± 3	70 ± 2	61 ± 1	46 ± 5	41 ± 4
Succinic acid	64 ± 1	68 ± 5	61 ± 3	47 ± 5	41 ± 2	62 ± 2	71 ± 4	60 ± 5	52 ± 2	44 ± 3
1,2,4-Benzenetriol	67 ± 5	64 ± 1	62 ± 5	42 ± 3	38 ± 4	69 ± 4	69 ± 3	63 ± 3	46 ± 4	42 ± 1
Hydroquinone	70 ± 1	63 ± 3	67 ± 4	44 ± 2	43 ± 5	70 ± 2	65 ± 4	60 ± 2	41 ± 3	38 ± 2
Sulfamethoxazole	66 ± 3	71 ± 1	64 ± 2	52 ± 5	45 ± 3	66 ± 5	70 ± 2	67 ± 5	47 ± 1	42 ± 5
p-Nitrophenol	64 ± 5	66 ± 4	67 ± 1	44 ± 2	43 ± 2	64 ± 3	67 ± 1	61 ± 4	40 ± 3	38 ± 4

[1] 100% methanol; [2] methanol-formic acid (98:2, v/v); [3] methanol-ammonia solution (95:5, v/v)

Table 5.4: Recoveries (%) and RSD (n=3) for different SPE materials at adjusted pH value (pH=2)

Compound	Oasis HLB	Oasis MAX	Oasis MCX	Oasis WAX	Oasis WCX	Strata-X	Strata-X-A	Strata-X-C	Strata-X-AW	Strata-X-CW
1H-Benzotriazole	74 ± 1	75 ± 3	71 ± 2	57 ± 2	51 ± 3	71 ± 2	73 ± 4	70 ± 1	55 ± 2	50 ± 3
Bisphenol A	77 ± 2	74 ± 2	75 ± 1	50 ± 4	45 ± 2	75 ± 4	76 ± 1	72 ± 3	52 ± 1	48 ± 2
Catechol	74 ± 1	73 ± 4	74 ± 3	49 ± 2	47 ± 3	78 ± 1	76 ± 3	71 ± 2	53 ± 3	45 ± 5
p-Benzoquinone	75 ± 2	74 ± 3	73 ± 2	50 ± 5	44 ± 4	76 ± 3	75 ± 5	70 ± 4	56 ± 2	51 ± 3
c,c-Muconic acid	72 ± 3	71 ± 1	68 ± 4	56 ± 2	49 ± 2	73 ± 2	74 ± 3	73 ± 1	54 ± 3	46 ± 1
t,t-Muconic acid	74 ± 4	73 ± 3	70 ± 2	50 ± 2	43 ± 3	71 ± 1	72 ± 2	71 ± 3	47 ± 1	41 ± 2
Carbamazepine	73 ± 2	70 ± 2	71 ± 2	54 ± 4	51 ± 2	77 ± 3	76 ± 4	70 ± 2	51 ± 4	46 ± 2
Anthranilic acid	74 ± 3	73 ± 3	75 ± 1	53 ± 1	47 ± 4	75 ± 4	72 ± 1	75 ± 3	55 ± 3	50 ± 3
Glyoxylic acid	73 ± 5	72 ± 3	64 ± 4	49 ± 2	46 ± 5	76 ± 3	70 ± 5	66 ± 4	54 ± 5	51 ± 4
Oxamic acid	70 ± 3	70 ± 1	68 ± 4	53 ± 3	50 ± 2	68 ± 5	68 ± 2	70 ± 1	52 ± 3	47 ± 2
Ciprofloxacin	73 ± 2	74 ± 2	82 ± 1	56 ± 2	51 ± 4	70 ± 3	70 ± 1	79 ± 2	53 ± 4	50 ± 1
Diclofenac	78 ± 4	73 ± 4	77 ± 3	52 ± 3	47 ± 2	76 ± 1	71 ± 2	70 ± 4	55 ± 2	51 ± 4
2,6-Dichloroaniline	72 ± 2	71 ± 2	80 ± 2	56 ± 5	51 ± 1	74 ± 2	70 ± 5	78 ± 3	52 ± 4	46 ± 3
Metoprolol	65 ± 1	72 ± 3	83 ± 4	58 ± 4	50 ± 3	67 ± 4	67 ± 2	81 ± 1	56 ± 3	47 ± 4
Paracetamol	77 ± 2	74 ± 4	72 ± 1	56 ± 2	52 ± 2	79 ± 2	76 ± 3	74 ± 2	50 ± 1	44 ± 2
Oxalic acid	71 ± 4	76 ± 3	69 ± 2	50 ± 2	46 ± 4	68 ± 3	78 ± 4	70 ± 3	54 ± 2	50 ± 5
Oxaloacetic acid	73 ± 3	78 ± 3	70 ± 2	52 ± 3	47 ± 5	70 ± 4	70 ± 2	68 ± 5	48 ± 4	44 ± 3
Malic acid	72 ± 2	75 ± 1	68 ± 3	54 ± 1	50 ± 3	76 ± 3	76 ± 1	72 ± 2	51 ± 5	47 ± 4
Malonic acid	74 ± 4	74 ± 2	73 ± 2	57 ± 5	48 ± 3	71 ± 5	72 ± 2	74 ± 4	54 ± 2	51 ± 5
Maleic acid	76 ± 3	75 ± 4	74 ± 3	53 ± 3	44 ± 1	78 ± 2	77 ± 4	72 ± 3	50 ± 3	45 ± 2
Succinic acid	77 ± 1	77 ± 2	76 ± 4	50 ± 1	46 ± 2	74 ± 3	75 ± 2	74 ± 3	52 ± 2	48 ± 4
1,2,4-Benzenetriol	79 ± 2	76 ± 1	69 ± 4	52 ± 5	43 ± 2	81 ± 5	77 ± 3	72 ± 4	47 ± 1	42 ± 3
Hydroquinone	76 ± 1	73 ± 3	72 ± 2	49 ± 4	47 ± 5	78 ± 4	74 ± 5	70 ± 3	51 ± 2	49 ± 4
Sulfamethoxazole	78 ± 4	71 ± 2	79 ± 1	53 ± 3	49 ± 1	75 ± 2	73 ± 1	75 ± 2	55 ± 2	52 ± 1
p-Nitrophenol	77 ± 3	75 ± 4	72 ± 2	56 ± 4	52 ± 3	74 ± 4	75 ± 3	70 ± 5	53 ± 1	50 ± 3

Table 5.5: Recoveries (%) and RSD (n=3) for different SPE materials at adjusted pH value (pH=5)

Compound	Oasis HLB	Oasis MAX	Oasis MCX	Oasis WAX	Oasis WCX	Strata-X	Strata-X-A	Strata-X-C	Strata-X-AW	Strata-X-CW
1H-Benzotriazole	72 ± 3	74 ± 2	77 ± 2	55 ± 4	50 ± 1	74 ± 1	72 ± 3	73 ± 1	52 ± 3	47 ± 2
Bisphenol A	78 ± 1	75 ± 1	76 ± 4	53 ± 1	51 ± 4	73 ± 4	74 ± 2	75 ± 3	55 ± 4	48 ± 3
Catechol	76 ± 4	72 ± 5	76 ± 2	58 ± 5	55 ± 2	74 ± 2	73 ± 4	74 ± 1	53 ± 3	50 ± 2
p-Benzoquinone	74 ± 2	78 ± 3	71 ± 5	50 ± 2	47 ± 5	72 ± 3	80 ± 1	68 ± 5	56 ± 2	53 ± 3
c,c-Muconic acid	70 ± 3	77 ± 2	70 ± 3	55 ± 1	53 ± 2	63 ± 2	78 ± 4	74 ± 2	51 ± 5	48 ± 1
t,t-Muconic acid	68 ± 1	79 ± 3	72 ± 1	53 ± 4	46 ± 3	64 ± 4	75 ± 3	71 ± 1	56 ± 2	54 ± 3
Carbamazepine	74 ± 2	73 ± 2	75 ± 3	52 ± 5	47 ± 4	75 ± 1	75 ± 4	72 ± 2	52 ± 3	45 ± 2
Anthranilic acid	73 ± 3	79 ± 2	72 ± 4	56 ± 3	51 ± 2	79 ± 3	79 ± 1	68 ± 2	55 ± 1	50 ± 4
Glyoxylic acid	63 ± 5	81 ± 3	70 ± 3	48 ± 2	45 ± 5	65 ± 5	77 ± 2	69 ± 3	52 ± 3	51 ± 2
Oxamic acid	67 ± 2	77 ± 4	76 ± 5	55 ± 2	50 ± 4	64 ± 4	76 ± 3	73 ± 5	50 ± 5	47 ± 2
Ciprofloxacin	75 ± 3	78 ± 2	83 ± 1	57 ± 3	50 ± 2	73 ± 1	78 ± 3	84 ± 2	47 ± 4	42 ± 1
Diclofenac	72 ± 2	77 ± 1	74 ± 3	53 ± 4	49 ± 1	70 ± 2	80 ± 1	70 ± 4	51 ± 2	50 ± 4
2,6-Dichloroaniline	76 ± 4	72 ± 3	70 ± 4	57 ± 1	51 ± 5	75 ± 4	71 ± 3	73 ± 5	57 ± 4	53 ± 3
Metoprolol	66 ± 3	70 ± 1	81 ± 2	52 ± 3	45 ± 1	68 ± 3	68 ± 5	82 ± 2	54 ± 2	49 ± 5
Paracetamol	75 ± 2	73 ± 3	73 ± 1	55 ± 4	52 ± 3	73 ± 4	73 ± 1	71 ± 3	51 ± 3	46 ± 4
Oxalic acid	64 ± 5	79 ± 5	68 ± 2	51 ± 5	47 ± 4	64 ± 5	77 ± 2	66 ± 1	54 ± 2	48 ± 5
Oxaloacetic acid	69 ± 3	77 ± 4	71 ± 2	58 ± 2	56 ± 5	66 ± 3	78 ± 5	73 ± 4	56 ± 5	52 ± 2
Malic acid	63 ± 4	80 ± 2	70 ± 5	54 ± 4	49 ± 2	69 ± 4	81 ± 1	71 ± 2	50 ± 3	46 ± 4
Malonic acid	67 ± 2	78 ± 3	71 ± 2	50 ± 3	47 ± 4	65 ± 1	75 ± 3	70 ± 4	52 ± 5	48 ± 2
Maleic acid	66 ± 4	79 ± 3	76 ± 3	52 ± 1	45 ± 3	64 ± 2	80 ± 4	72 ± 1	48 ± 3	40 ± 4
Succinic acid	72 ± 2	76 ± 1	73 ± 4	56 ± 3	50 ± 2	69 ± 5	74 ± 1	75 ± 3	55 ± 4	47 ± 3
1,2,4-Benzenetriol	76 ± 4	73 ± 2	70 ± 4	54 ± 4	51 ± 5	73 ± 2	76 ± 3	73 ± 5	58 ± 5	53 ± 3
Hydroquinone	79 ± 3	74 ± 5	74 ± 2	49 ± 5	47 ± 2	77 ± 3	75 ± 5	69 ± 4	53 ± 3	50 ± 5
Sulfamethoxazole	75 ± 1	75 ± 2	69 ± 4	53 ± 3	49 ± 1	73 ± 2	77 ± 4	72 ± 3	54 ± 1	46 ± 3
p-Nitrophenol	78 ± 4	73 ± 3	74 ± 3	55 ± 4	50 ± 3	75 ± 1	73 ± 3	72 ± 5	50 ± 3	45 ± 2

Table 5.6: Recoveries (%) and RSD (n=3) for different SPE materials at adjusted pH value (pH=7)

Compound	Oasis HLB	Oasis MAX	Oasis MCX	Oasis WAX	Oasis WCX	Strata-X	Strata-X-A	Strata-X-C	Strata-X-AW	Strata-X-CW
1H-Benzotriazole	76 ± 4	75 ± 3	72 ± 1	55 ± 1	53 ± 3	73 ± 2	73 ± 4	70 ± 3	52 ± 3	50 ± 2
Bisphenol A	74 ± 3	74 ± 1	73 ± 3	52 ± 4	48 ± 2	77 ± 3	71 ± 1	73 ± 2	55 ± 1	51 ± 4
Catechol	75 ± 5	71 ± 4	74 ± 4	51 ± 2	49 ± 1	75 ± 1	74 ± 3	70 ± 4	50 ± 5	46 ± 1
p-Benzoquinone	72 ± 2	73 ± 5	70 ± 3	56 ± 4	53 ± 4	77 ± 3	75 ± 2	75 ± 5	52 ± 2	50 ± 3
c,c-Muconic acid	68 ± 1	78 ± 3	71 ± 1	52 ± 3	50 ± 2	62 ± 2	79 ± 4	70 ± 1	52 ± 4	46 ± 1
t,t-Muconic acid	63 ± 2	80 ± 4	70 ± 3	51 ± 4	48 ± 1	60 ± 4	80 ± 3	72 ± 4	48 ± 2	45 ± 5
Carbamazepine	75 ± 3	74 ± 1	73 ± 2	48 ± 1	43 ± 3	77 ± 1	77 ± 4	71 ± 2	54 ± 1	47 ± 1
Anthranilic acid	72 ± 2	80 ± 3	69 ± 2	45 ± 3	42 ± 3	78 ± 3	77 ± 2	66 ± 5	53 ± 2	44 ± 4
Glyoxylic acid	60 ± 3	82 ± 4	67 ± 4	54 ± 2	50 ± 5	58 ± 3	79 ± 4	68 ± 3	51 ± 3	49 ± 3
Oxamic acid	65 ± 2	78 ± 2	70 ± 5	56 ± 3	52 ± 2	68 ± 5	81 ± 2	71 ± 4	55 ± 2	51 ± 4
Ciprofloxacin	73 ± 2	79 ± 3	80 ± 3	55 ± 2	50 ± 3	73 ± 3	80 ± 4	77 ± 2	57 ± 4	53 ± 2
Diclofenac	69 ± 4	78 ± 1	76 ± 3	56 ± 1	53 ± 1	71 ± 2	79 ± 5	75 ± 3	59 ± 2	57 ± 3
2,6-Dichloroaniline	74 ± 2	69 ± 1	71 ± 4	50 ± 3	44 ± 3	77 ± 5	72 ± 3	72 ± 1	57 ± 4	52 ± 2
Metoprolol	66 ± 3	71 ± 2	80 ± 2	54 ± 3	46 ± 2	70 ± 1	72 ± 3	80 ± 2	58 ± 3	50 ± 4
Paracetamol	78 ± 1	71 ± 4	70 ± 3	55 ± 2	50 ± 1	75 ± 2	71 ± 4	73 ± 3	53 ± 1	47 ± 4
Oxalic acid	59 ± 3	78 ± 5	73 ± 5	53 ± 5	48 ± 4	63 ± 4	81 ± 2	70 ± 3	54 ± 5	50 ± 3
Oxaloacetic acid	64 ± 4	79 ± 4	70 ± 2	52 ± 2	46 ± 5	62 ± 3	77 ± 5	68 ± 1	55 ± 4	52 ± 1
Malic acid	61 ± 2	77 ± 2	71 ± 3	55 ± 3	49 ± 2	63 ± 5	80 ± 3	72 ± 2	50 ± 4	45 ± 5
Malonic acid	66 ± 4	76 ± 3	72 ± 3	49 ± 5	44 ± 4	62 ± 3	77 ± 1	75 ± 4	53 ± 2	51 ± 1
Maleic acid	67 ± 1	78 ± 1	74 ± 2	53 ± 2	50 ± 2	66 ± 2	79 ± 4	70 ± 5	47 ± 5	42 ± 4
Succinic acid	70 ± 3	79 ± 2	75 ± 4	52 ± 4	48 ± 1	68 ± 4	76 ± 3	76 ± 1	54 ± 1	48 ± 5
1,2,4-Benzenetriol	77 ± 5	75 ± 4	73 ± 2	54 ± 5	51 ± 4	76 ± 5	75 ± 2	72 ± 4	55 ± 2	54 ± 3
Hydroquinone	75 ± 3	71 ± 2	77 ± 3	58 ± 3	55 ± 5	78 ± 3	77 ± 4	72 ± 3	53 ± 2	47 ± 3
Sulfamethoxazole	71 ± 4	79 ± 1	71 ± 2	51 ± 2	46 ± 1	69 ± 2	79 ± 3	73 ± 4	55 ± 3	46 ± 1
p-Nitrophenol	73 ± 3	76 ± 4	72 ± 1	55 ± 3	52 ± 4	73 ± 4	75 ± 2	71 ± 3	51 ± 1	50 ± 3

Table 5.7: Recoveries (%) and RSD (n=3) for different SPE materials at adjusted pH value (pH=9)

Compound	Oasis HLB	Oasis MAX	Oasis MCX	Oasis WAX	Oasis WCX	Strata-X	Strata-X-A	Strata-X-C	Strata-X-AW	Strata-X-CW
1H-Benzotriazole	73 ± 1	75 ± 4	73 ± 3	55 ± 4	49 ± 2	70 ± 4	75 ± 2	71 ± 3	56 ± 1	51 ± 4
Bisphenol A	78 ± 2	77 ± 1	71 ± 4	53 ± 2	51 ± 1	75 ± 3	76 ± 1	72 ± 2	55 ± 3	52 ± 1
Catechol	78 ± 1	76 ± 2	70 ± 2	50 ± 3	47 ± 3	80 ± 1	79 ± 3	68 ± 4	57 ± 2	51 ± 3
p-Benzoquinone	71 ± 4	72 ± 3	73 ± 1	58 ± 1	51 ± 2	75 ± 2	72 ± 5	76 ± 1	55 ± 4	50 ± 4
c,c-Muconic acid	66 ± 3	80 ± 5	72 ± 2	53 ± 2	46 ± 3	63 ± 5	81 ± 3	72 ± 2	47 ± 3	42 ± 4
t,t-Muconic acid	64 ± 2	78 ± 3	68 ± 5	48 ± 1	43 ± 2	66 ± 2	80 ± 1	70 ± 3	56 ± 3	51 ± 4
Carbamazepine	73 ± 2	72 ± 4	75 ± 3	54 ± 2	47 ± 1	75 ± 4	75 ± 3	73 ± 1	48 ± 2	43 ± 3
Anthranilic acid	67 ± 3	83 ± 5	67 ± 1	50 ± 4	44 ± 2	79 ± 1	81 ± 2	65 ± 2	51 ± 2	48 ± 3
Glyoxylic acid	63 ± 4	80 ± 2	69 ± 3	53 ± 3	49 ± 2	67 ± 3	77 ± 5	66 ± 4	50 ± 2	43 ± 5
Oxamic acid	66 ± 2	76 ± 4	74 ± 3	50 ± 1	46 ± 3	63 ± 4	73 ± 2	71 ± 3	56 ± 2	50 ± 1
Ciprofloxacin	70 ± 3	81 ± 2	78 ± 5	57 ± 5	53 ± 1	75 ± 1	82 ± 3	80 ± 2	52 ± 1	47 ± 3
Diclofenac	67 ± 2	82 ± 4	74 ± 3	56 ± 2	52 ± 4	66 ± 2	78 ± 4	74 ± 3	54 ± 1	49 ± 2
2,6-Dichloroaniline	76 ± 5	68 ± 3	73 ± 2	53 ± 4	49 ± 3	76 ± 2	70 ± 3	73 ± 1	50 ± 3	46 ± 1
Metoprolol	72 ± 3	70 ± 1	76 ± 4	50 ± 2	49 ± 5	74 ± 3	70 ± 2	79 ± 3	56 ± 1	52 ± 3
Paracetamol	75 ± 4	75 ± 2	73 ± 1	52 ± 3	45 ± 4	73 ± 2	77 ± 4	71 ± 1	57 ± 2	51 ± 1
Oxalic acid	60 ± 2	79 ± 4	67 ± 2	54 ± 4	50 ± 1	61 ± 4	82 ± 1	70 ± 3	51 ± 3	45 ± 2
Oxaloacetic acid	65 ± 4	76 ± 2	70 ± 5	59 ± 3	53 ± 2	60 ± 3	75 ± 5	71 ± 2	55 ± 1	50 ± 4
Malic acid	68 ± 3	78 ± 5	69 ± 2	51 ± 5	47 ± 5	65 ± 5	79 ± 3	73 ± 2	53 ± 2	45 ± 2
Malonic acid	63 ± 5	80 ± 2	70 ± 1	54 ± 2	46 ± 1	66 ± 3	80 ± 2	72 ± 5	56 ± 4	50 ± 3
Maleic acid	66 ± 2	79 ± 4	74 ± 3	55 ± 4	49 ± 2	65 ± 2	77 ± 4	71 ± 3	52 ± 1	49 ± 1
Succinic acid	68 ± 3	80 ± 1	74 ± 3	53 ± 2	51 ± 4	66 ± 3	80 ± 4	75 ± 1	55 ± 3	50 ± 1
1,2,4-Benzenetriol	74 ± 4	74 ± 2	70 ± 5	51 ± 2	50 ± 1	75 ± 5	76 ± 3	71 ± 4	57 ± 3	53 ± 4
Hydroquinone	77 ± 3	75 ± 5	72 ± 2	54 ± 1	48 ± 3	75 ± 4	73 ± 2	73 ± 3	58 ± 5	54 ± 2
Sulfamethoxazole	65 ± 4	78 ± 2	73 ± 4	45 ± 2	40 ± 2	63 ± 2	75 ± 4	69 ± 2	48 ± 4	47 ± 3
p-Nitrophenol	68 ± 2	77 ± 4	75 ± 3	52 ± 1	49 ± 3	65 ± 2	79 ± 3	72 ± 5	55 ± 5	51 ± 4

Table 5.8: Recoveries (%) and RSD (n=3) for different SPE materials at adjusted pH value (pH=12)

Compound	Oasis HLB	Oasis MAX	Oasis MCX	Oasis WAX	Oasis WCX	Strata-X	Strata-X-A	Strata-X-C	Strata-X-AW	Strata-X-CW
1H-Benzotriazole	71 ± 1	76 ± 3	71 ± 2	56 ± 3	50 ± 3	68 ± 2	78 ± 4	72 ± 1	50 ± 2	47 ± 3
Bisphenol A	68 ± 3	80 ± 2	70 ± 4	52 ± 2	49 ± 2	66 ± 1	77 ± 3	74 ± 2	47 ± 1	41 ± 2
Catechol	72 ± 5	75 ± 1	72 ± 3	57 ± 5	49 ± 5	69 ± 2	75 ± 4	70 ± 5	55 ± 3	50 ± 4
p-Benzoquinone	73 ± 1	70 ± 4	75 ± 3	54 ± 3	46 ± 3	77 ± 3	75 ± 1	74 ± 4	51 ± 2	45 ± 3
c,c-Muconic acid	66 ± 4	81 ± 2	70 ± 5	52 ± 1	47 ± 2	61 ± 1	79 ± 3	73 ± 1	57 ± 4	51 ± 1
t,t-Muconic acid	65 ± 2	80 ± 5	71 ± 3	50 ± 2	45 ± 4	59 ± 2	78 ± 1	70 ± 3	52 ± 2	50 ± 3
Carbamazepine	77 ± 3	68 ± 2	77 ± 2	57 ± 4	54 ± 3	80 ± 4	74 ± 3	75 ± 1	51 ± 3	44 ± 2
Anthranilic acid	69 ± 2	81 ± 4	68 ± 1	54 ± 3	50 ± 5	77 ± 3	80 ± 5	69 ± 3	54 ± 5	46 ± 1
Glyoxylic acid	67 ± 4	82 ± 3	70 ± 5	53 ± 2	46 ± 4	69 ± 2	83 ± 5	72 ± 4	55 ± 1	50 ± 4
Oxamic acid	66 ± 2	80 ± 5	72 ± 3	55 ± 2	48 ± 5	67 ± 4	77 ± 3	71 ± 4	52 ± 4	48 ± 3
Ciprofloxacin	71 ± 1	80 ± 3	72 ± 4	50 ± 2	49 ± 3	72 ± 3	78 ± 1	70 ± 2	56 ± 3	51 ± 1
Diclofenac	65 ± 3	81 ± 2	76 ± 3	56 ± 1	54 ± 2	69 ± 2	80 ± 4	74 ± 3	49 ± 2	45 ± 3
2,6-Dichloroaniline	74 ± 2	69 ± 4	75 ± 2	57 ± 3	53 ± 2	78 ± 3	71 ± 4	70 ± 1	53 ± 2	47 ± 5
Metoprolol	77 ± 4	73 ± 1	71 ± 4	54 ± 3	48 ± 1	75 ± 5	73 ± 2	68 ± 3	56 ± 4	52 ± 1
Paracetamol	71 ± 2	80 ± 3	75 ± 2	55 ± 2	51 ± 2	68 ± 2	78 ± 1	70 ± 4	51 ± 4	44 ± 2
Oxalic acid	65 ± 1	76 ± 5	65 ± 3	58 ± 3	50 ± 4	60 ± 4	80 ± 2	67 ± 5	53 ± 2	45 ± 4
Oxaloacetic acid	64 ± 4	80 ± 3	69 ± 1	53 ± 5	46 ± 5	67 ± 3	76 ± 1	71 ± 4	51 ± 3	49 ± 2
Malic acid	66 ± 2	81 ± 1	65 ± 5	50 ± 3	45 ± 3	64 ± 1	78 ± 4	74 ± 5	48 ± 5	43 ± 4
Malonic acid	65 ± 5	78 ± 4	72 ± 2	52 ± 5	43 ± 2	63 ± 3	82 ± 2	67 ± 3	52 ± 2	48 ± 5
Maleic acid	67 ± 3	76 ± 2	70 ± 4	51 ± 2	50 ± 3	66 ± 2	78 ± 5	65 ± 4	55 ± 3	47 ± 2
Succinic acid	65 ± 2	81 ± 3	76 ± 5	56 ± 4	52 ± 2	67 ± 4	78 ± 3	75 ± 2	51 ± 2	48 ± 3
1,2,4-Benzenetriol	71 ± 5	76 ± 2	72 ± 4	51 ± 3	47 ± 5	68 ± 5	79 ± 1	73 ± 4	54 ± 4	51 ± 3
Hydroquinone	72 ± 4	77 ± 2	70 ± 3	57 ± 5	51 ± 4	70 ± 2	75 ± 4	72 ± 3	50 ± 2	47 ± 5
Sulfamethoxazole	68 ± 1	80 ± 3	71 ± 2	54 ± 4	51 ± 1	64 ± 3	81 ± 4	67 ± 3	52 ± 1	45 ± 2
p-Nitrophenol	70 ± 3	79 ± 5	70 ± 2	55 ± 2	53 ± 4	66 ± 1	76 ± 3	68 ± 1	53 ± 3	49 ± 1

Table 5.9: Recoveries (%) and RSD (n=3) for the developed tandem configuration of both Oasis and Strata families at different pH values

Compound	pH 2		pH 5		pH 7		pH 9		pH 12	
	Oasis	Strata	Oasis	Strata	Oasis	Strata	Oasis	Strata	Oasis	Strata
1H-Benzotriazole	95 ± 5	91 ± 2	95 ± 2	94 ± 4	95 ± 5	90 ± 3	95 ± 6	91 ± 4	92 ± 4	94 ± 3
Bisphenol A	94 ± 4	94 ± 5	94 ± 4	92 ± 2	90 ± 2	92 ± 4	94 ± 3	92 ± 2	95 ± 3	91 ± 4
Catechol	92 ± 3	92 ± 2	92 ± 6	93 ± 6	92 ± 3	91 ± 3	92 ± 5	96 ± 5	93 ± 6	90 ± 3
p-Benzoquinone	94 ± 5	91 ± 6	93 ± 2	92 ± 5	93 ± 6	93 ± 5	91 ± 2	92 ± 3	95 ± 4	92 ± 6
c,c-Muconic acid	90 ± 4	91 ± 3	96 ± 4	94 ± 3	91 ± 2	92 ± 2	93 ± 4	94 ± 4	94 ± 6	92 ± 4
t,t-Muconic acid	91 ± 4	93 ± 5	94 ± 5	90 ± 5	95 ± 4	94 ± 5	97 ± 2	95 ± 6	91 ± 3	94 ± 3
Carbamazepine	92 ± 2	92 ± 6	94 ± 2	94 ± 3	90 ± 5	90 ± 3	94 ± 3	93 ± 3	93 ± 5	95 ± 5
Anthranilic acid	94 ± 3	93 ± 2	97 ± 3	91 ± 4	92 ± 3	93 ± 6	96 ± 5	94 ± 6	92 ± 3	93 ± 6
Glyoxylic acid	91 ± 6	93 ± 4	93 ± 6	90 ± 5	95 ± 4	91 ± 6	92 ± 3	90 ± 5	93 ± 6	95 ± 5
Oxamic acid	90 ± 4	91 ± 3	91 ± 3	90 ± 3	90 ± 6	95 ± 3	90 ± 4	91 ± 3	95 ± 2	91 ± 3
Ciprofloxacin	92 ± 3	90 ± 5	97 ± 2	94 ± 5	93 ± 3	96 ± 5	95 ± 2	94 ± 4	93 ± 4	94 ± 5
Diclofenac	95 ± 3	92 ± 3	94 ± 4	92 ± 2	95 ± 5	98 ± 3	96 ± 6	92 ± 2	95 ± 5	93 ± 3
2,6-Dichloroaniline	93 ± 4	91 ± 6	95 ± 3	95 ± 6	92 ± 6	94 ± 5	94 ± 4	93 ± 4	90 ± 3	91 ± 5
Metoprolol	94 ± 3	96 ± 4	93 ± 5	94 ± 5	95 ± 3	96 ± 3	93 ± 2	95 ± 5	91 ± 5	93 ± 3
Paracetamol	96 ± 3	92 ± 2	95 ± 2	92 ± 3	96 ± 5	92 ± 4	90 ± 5	92 ± 3	94 ± 2	92 ± 2
Oxalic acid	91 ± 5	93 ± 6	92 ± 6	94 ± 6	92 ± 6	93 ± 6	92 ± 6	95 ± 6	92 ± 5	93 ± 5
Oxaloacetic acid	94 ± 6	91 ± 5	94 ± 4	94 ± 4	94 ± 4	90 ± 4	90 ± 5	91 ± 4	94 ± 6	91 ± 6
Malic acid	90 ± 4	94 ± 6	91 ± 3	92 ± 6	90 ± 4	91 ± 5	91 ± 3	93 ± 6	90 ± 4	94 ± 3
Malonic acid	93 ± 3	91 ± 2	90 ± 4	90 ± 5	93 ± 2	90 ± 2	90 ± 5	91 ± 4	92 ± 3	94 ± 5
Maleic acid	91 ± 6	93 ± 3	93 ± 5	94 ± 4	90 ± 5	92 ± 4	95 ± 4	90 ± 3	91 ± 5	92 ± 6
Succinic acid	92 ± 4	90 ± 2	92 ± 2	90 ± 3	92 ± 3	93 ± 6	93 ± 2	95 ± 5	92 ± 2	90 ± 4
1,2,4-Benzenetriol	92 ± 5	93 ± 6	90 ± 4	91 ± 5	91 ± 6	90 ± 4	90 ± 6	92 ± 6	95 ± 4	93 ± 5
Hydroquinone	90 ± 4	92 ± 5	91 ± 5	93 ± 6	93 ± 3	94 ± 3	92 ± 4	90 ± 4	94 ± 6	92 ± 3
Sulfamethoxazole	94 ± 3	93 ± 2	95 ± 3	92 ± 2	95 ± 5	92 ± 2	95 ± 2	93 ± 3	95 ± 4	96 ± 2
p-Nitrophenol	92 ± 5	90 ± 4	90 ± 6	91 ± 6	92 ± 4	90 ± 5	93 ± 5	95 ± 6	92 ± 3	90 ± 5

Table 5.10: Concentration levels (ng/L) of the compounds of interest in water matrices sampled in April 2014 and February 2015 from wastewater treatment plant (WWTP) and Ruhr river in Germany

Compound	2014				2015			
	$WWBO_3$	$WWAO_3$	WWFE	SW	$WWBO_3$	$WWAO_3$	WWFE	SW
1H-Benzotriazole	1950	742	395	87	2340	816	472	110
Bisphenol A	391	143	52	24	227	94	43	26
Catechol	39	142	56	24	27	88	43	21
p-Benzoquinone	21	64	41	ND	ND	40	29	22
c,c-Muconic acid	ND	38	ND	25	ND	33	23	ND
t,t-Muconic acid	ND	40	ND	21	ND	28	ND	ND
Carbamazepine	3020	995	602	129	3410	1190	652	166
Anthranilic acid	24	85	39	23	34	55	32	22
Glyoxylic acid	ND	35	25	ND	ND	ND	ND	ND
Oxamic acid	22	37	25	ND	ND	28	ND	ND
Ciprofloxacin	418	145	70	26	351	119	48	23
Diclofenac	2540	936	501	95	3050	1090	616	147
2,6-Dichloroaniline	ND	57	30	ND	ND	29	23	ND
Metoprolol	2390	881	464	106	2680	1040	542	132
Paracetamol	719	218	128	35	937	341	179	43
Oxalic acid	24	67	ND	ND	39	88	33	26
Oxaloacetic acid	ND	50	24	ND	ND	ND	ND	ND
Malic acid	25	126	67	23	76	214	113	41
Malonic acid	ND	ND	ND	ND	ND	35	22	ND
Maleic acid	47	173	94	24	65	154	71	38
Succinic acid	37	184	104	30	68	102	88	35
1,2,4-Benzenetriol	ND	47	24	ND	ND	32	ND	24
Hydroquinone	ND	ND	ND	ND	ND	26	ND	ND
Sulfamethoxazole	1480	457	287	70	1160	409	231	57
p-Nitrophenol	25	81	36	26	44	131	64	27

SW: Surface water; $WWBO_3$: Wastewater before ozonation; $WWAO_3$: Wastewater after ozonation; WWFE: Wastewater final effluent

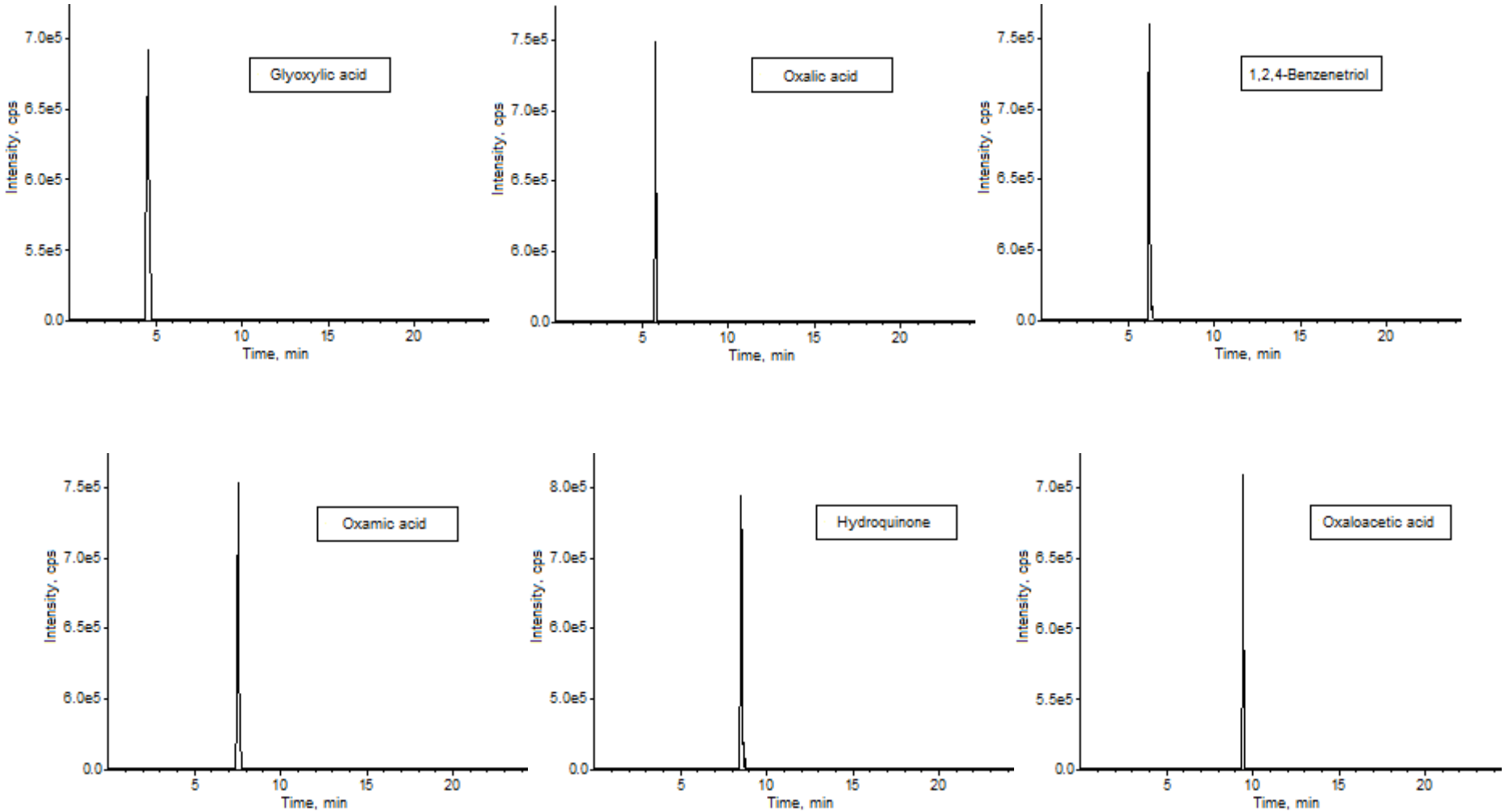


Figure 5.1: LC-ESI-MS/MS chromatograms for 25 analytes in spiked surface water

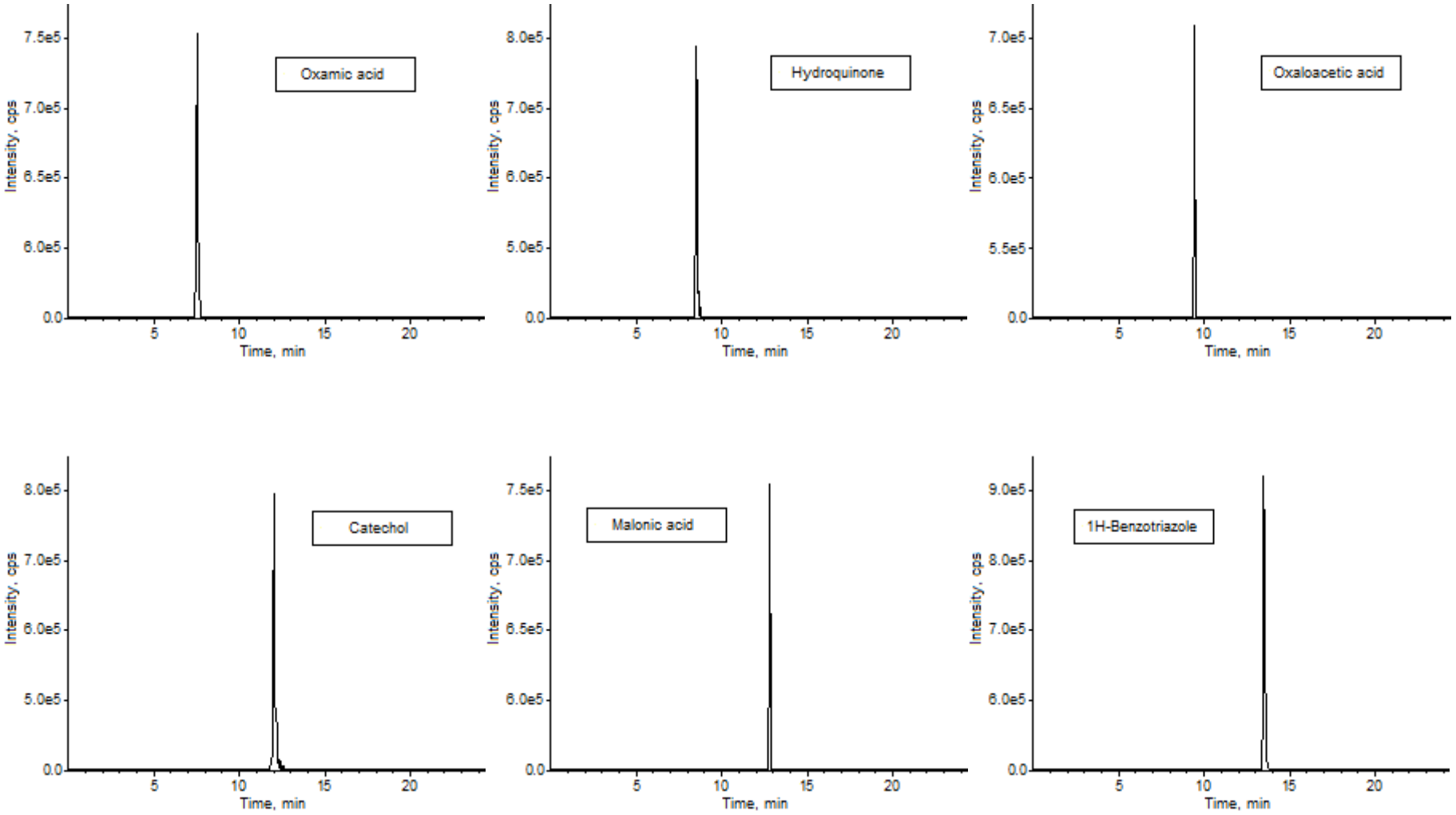


Figure 5.1: LC-ESI-MS/MS chromatograms for 25 analytes in spiked surface water (continued)

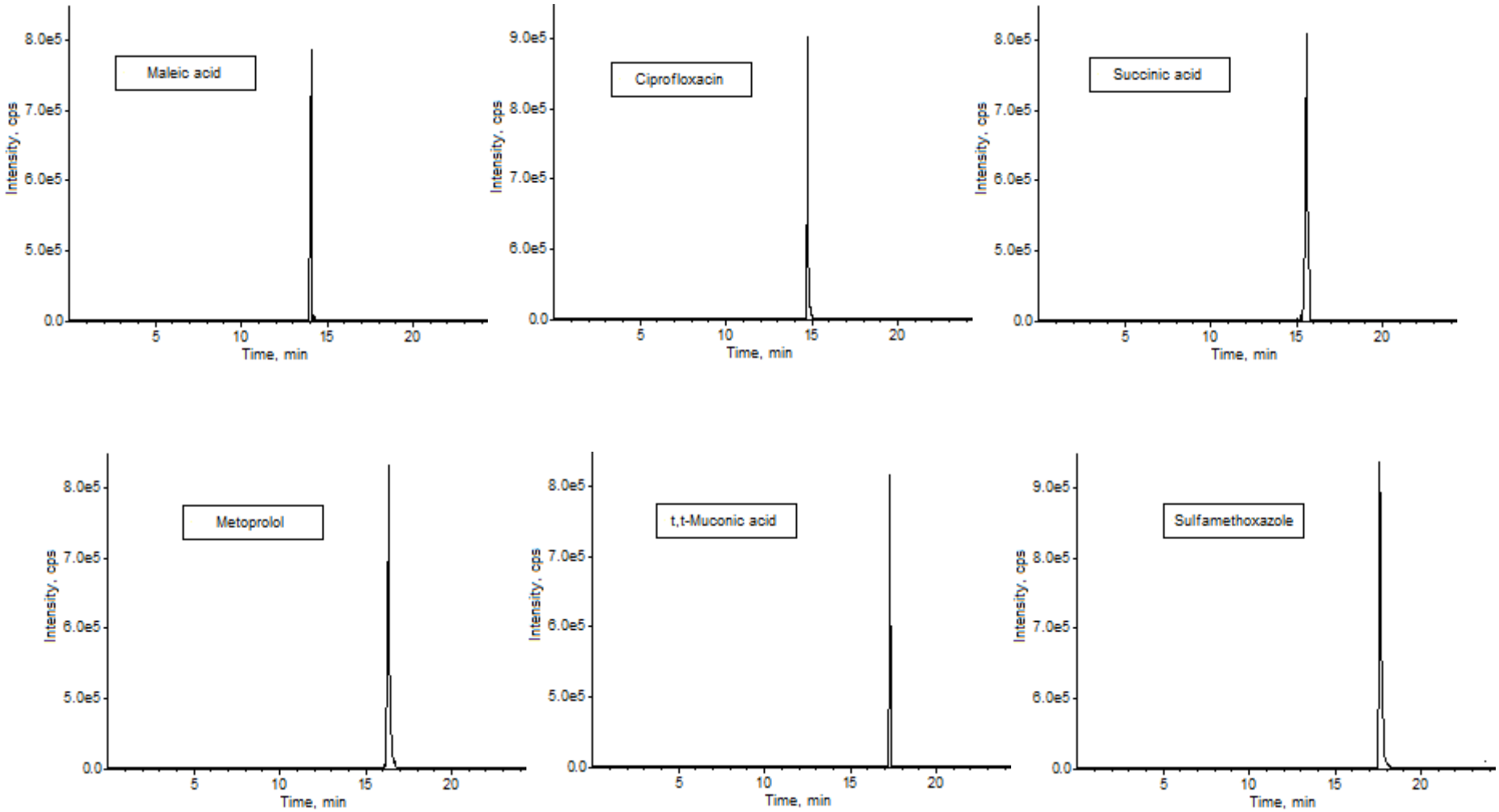


Figure 5.1: LC-ESI-MS/MS chromatograms for 25 analytes in spiked surface water (continued)

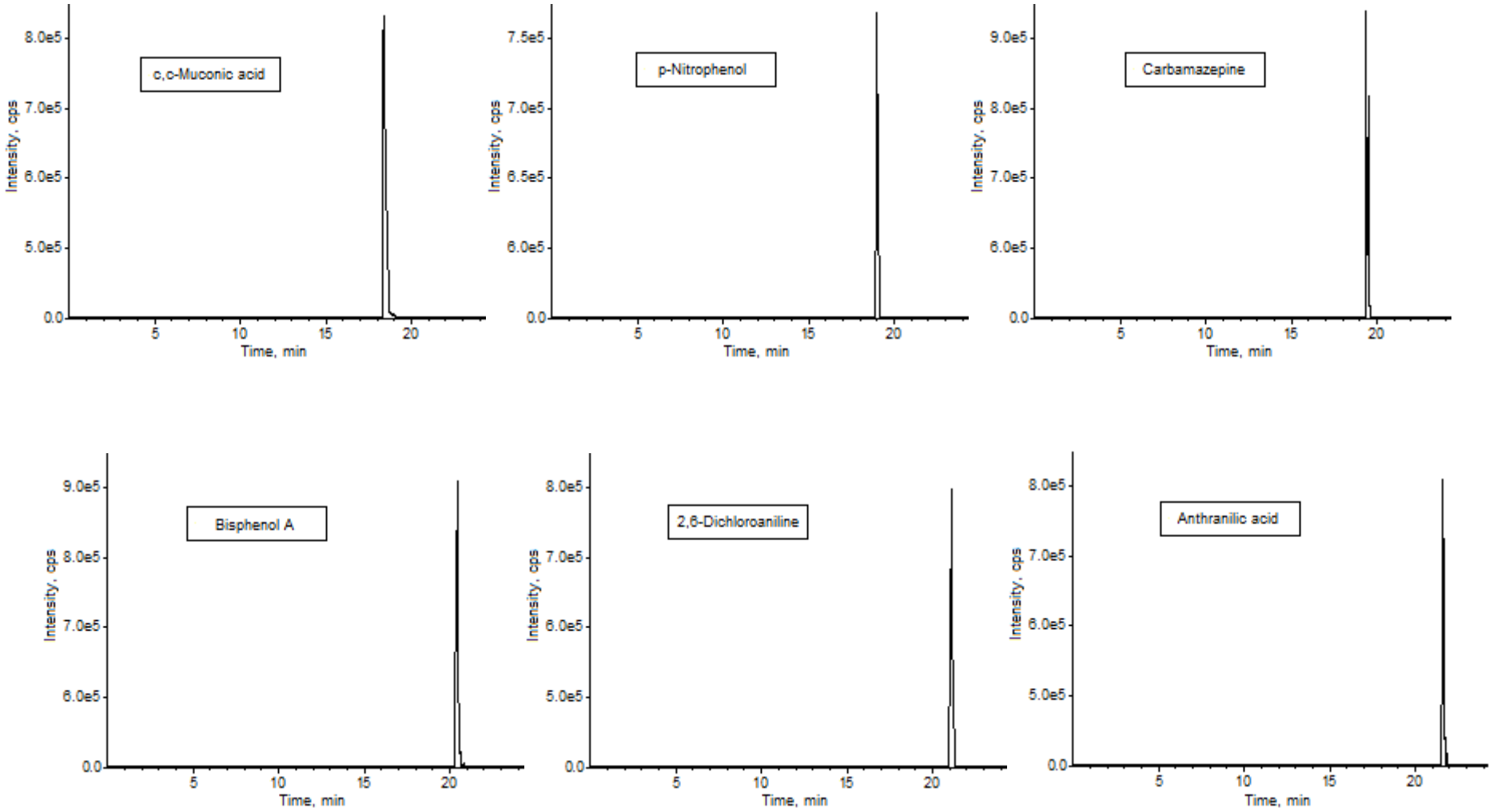


Figure 5.1: LC-ESI-MS/MS chromatograms for 25 analytes in spiked surface water (continued)

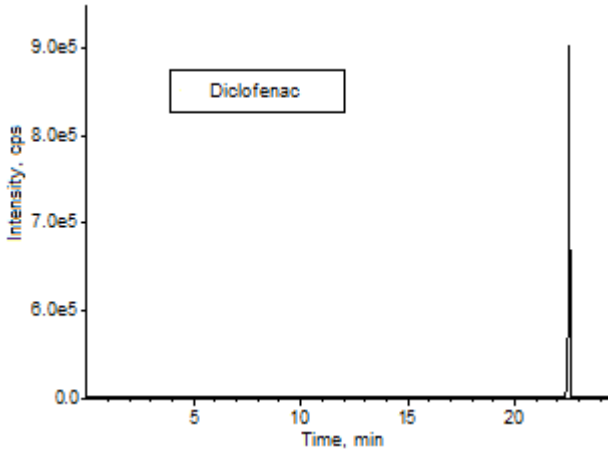


Figure 5.1: LC-ESI-MS/MS chromatograms for 25 analytes in spiked surface water (continued)

5.3 Suspect screening of micropollutants and their transformation products in advanced wastewater treatment

Table 5.11: Suspect list including compounds, uses, lab study water matrices, analytical methods, analytical columns, and the used mobile phases obtained from literatures [1-32]

Compound	Uses	Lab study (water matrix)	Analytical method	Analytical column	Mobile phase	RT (min) [literature]	Ref.
Bisphenol A (BPA)	Industry	Pure water	LC-UV, LC–MS and MS/MS	Uptispher HDO C18 (3.0 x 250 mm, 5 µm)	MeOH/H_2O	45.11	[1]
BPA-TP1	Ozonated TP					11.47	[1]
BPA-TP2	Ozonated TP					28.46	[1]
BPA-TP3	Ozonated TP					41.98	[1]
BPA-TP4	Ozonated TP						[1]
BPA-TP5	Ozonated TP						[1]
Caffeine (CAFF)	Psychoactive drug	Pure water	LC-TOF-MS	ZORBAX, SB-C18 (3.0 x 250 mm, 5 µm)	ACN/H_2O	NA	[2]
CAFF-TP1	Ozonated TP					NA	[2]
CAFF-TP2	Ozonated TP					NA	[2]
CAFF-TP3	Ozonated TP						[2]
CAFF-TP4	Ozonated TP					NA	[2]
CAFF-TP5	Ozonated TP					NA	[2]
CAFF-TP6	Ozonated TP					NA	[2]
Estrone sulfate (EST-S)	Steroid hormone	DWTP	LC-LTQ Orbitrap-MS	Gemini C18 (2.0 x 50 mm, 3 µm)	ACN/H_2O	9.25	[3]
EST-S-TP1	Ozonated TP					6.71	[3]
EST-S-TP2	Ozonated TP					6.78	[3]
EST-S-TP3	Ozonated TP					6.92	[3]
EST-S-TP4	Ozonated TP					7.55	[3]
EST-S-TP5	Ozonated TP					7.57	[3]
EST-S-TP6	Ozonated TP					7.74	[3]
EST-S-TP7	Ozonated TP					8.00	[3]
EST-S-TP8	Ozonated TP					8.13	[3]
EST-S-TP9	Ozonated TP					8.50	[3]
Trimethoprim (TMP)	Antibiotic	Pure water	LC-MS-MS	TC-C18 (4.6 x 150 mm, 5 µm)	MeOH/H_2O	15.41	[4]

Table 5.11: Suspect list including compounds, uses, lab study water matrices, analytical methods, analytical columns, and the used mobile phases obtained from literatures [1-32] (continued)

TMP-TP1	Ozonated TP					9.45	[4]
TMP-TP2	Ozonated TP						[4]
TMP-TP3	Ozonated TP					12.22	[4]
TMP-TP4	Ozonated TP						[4]
TMP-TP5	Ozonated TP					11.88	[4]
TMP-TP6	Ozonated TP						[4]
TMP-TP7	Ozonated TP					5.54	[4]
TMP-TP8	Ozonated TP					8.16	[4]
TMP-TP9	Ozonated TP						[4]
TMP-TP10	Ozonated TP					9.00	[4]
TMP-TP11	Ozonated TP					14.01	[4]
TMP-TP12	Ozonated TP					11.05	[4]
TMP-TP13	Ozonated TP						[4]
TMP-TP14	Ozonated TP					9.34	[4]
TMP-TP15	Ozonated TP					2.72	[4]
Roxithromycin (ROX)	Antibiotic	Pure water, sewage effluent	UPLC-Q-TOF-MS	ACQUITY BEH C18 (2.1 x 10 mm, 1.7 µm)	ACN-MeOH/H_2O	6.40	[5]
ROX-TP1	Ozonated TP					6.00	[5]
ROX-TP2	Ozonated TP					6.35	[5]
ROX-TP3	Ozonated TP					6.50	[5]
ROX-TP4	Ozonated TP					7.35	[5]
ROX-TP5	Ozonated TP					6.70	[5]
Methylbenzotriazole (MBZ)	Anticorrosive	DWTP	HPLC-Q-TOF-MS, HPLC-MS/MS, HPTLC	Zorbax Eclipse XDB-C18 (2.1 x 100 mm, 1.8 µm)	MeOH/H_2O	NA	[6]
MBZ-TP1	Ozonated TP					5.70	[6]
MBZ-TP2	Ozonated TP					9.50	[6]
MBZ-TP3	Ozonated TP					9.00	[6]
MBZ-TP4	Ozonated TP					5.40	[6]

Table 5.11: Suspect list including compounds, uses, lab study water matrices, analytical methods, analytical columns, and the used mobile phases obtained from literatures [1-32] (continued)

MBZ-TP5	Ozonated TP					8.60	[6]
MBZ-TP6	Ozonated TP						[6]
MBZ-TP7	Ozonated TP						[6]
MBZ-TP8	Ozonated TP						[6]
MBZ-TP9	Ozonated TP						[6]
MBZ-TP10	Ozonated TP						[6]
MBZ-TP11	Ozonated TP						[6]
Imazalil (IMZ)	Fungicide	Pure water, WWTP effluent	LC-LTQ Orbitrap-MS	Hypersil Gold aQ C18 (2.1 x 150 mm, 5.0 µm)	MeOH/H_2O	12.50	[7]
IMZ-TP1	Ozonated TP					6.17	[7]
IMZ-TP2	Ozonated TP						[7]
IMZ-TP3	Ozonated TP					6.03	[7]
IMZ-TP4	Ozonated TP					4.66	[7]
Ketoprofen (KPR)	Anti-inflammatory	Pure water	LC-UV, LC–MS	LiChroCART C18 (125 mm, 4.5 µm)	ACN/H_2O	4.20	[8]
KPR-TP1	Ozonated TP						[8]
KPR-TP2	Ozonated TP					3.90	[8]
KPR-TP3	Ozonated TP					4.90	[8]
KPR-TP4	Ozonated TP					6.20	[8]
Levofloxacin (LVX)	Antibiotic	Pure water	LC-UV, LC–MS	Luna C18 (3.0x150 mm, 3.0 µm)	MeOH/H_2O	20.88	[9]
LVX-TP1	Ozonated TP						[9]
LVX-TP2	Ozonated TP					17.69	[9]
LVX-TP3	Ozonated TP					21.77	[9]
LVX-TP4	Ozonated TP					9.45	[9]
LVX-TP5	Ozonated TP						[9]
LVX-TP6	Ozonated TP					27.73	[9]
LVX-TP7	Ozonated TP					41.47	[9]
Chlorophene (CLP)	Biocide	Pure water	LC-TOF-MS	XDB-C18 (4.6 x 50 mm, 1.8 µm)	ACN/H_2O	17.20	[10]

Table 5.11: Suspect list including compounds, uses, lab study water matrices, analytical methods, analytical columns, and the used mobile phases obtained from literatures [1-32] (continued)

CLP-TP1	Ozonated TP					11.66	[10]
CLP-TP2	Ozonated TP					15.02	[10]
CLP-TP3	Ozonated TP						[10]
CLP-TP4	Ozonated TP					9.94	[10]
CLP-TP5	Ozonated TP					10.20	[10]
CLP-TP6	Ozonated TP					8.74	[10]
CLP-TP7	Ozonated TP						[10]
CLP-TP8	Ozonated TP					7.75	[10]
CLP-TP9	Ozonated TP					8.43	[10]
CLP-TP10	Ozonated TP					10.05	[10]
Acyclovir (ACV)	Antiviral	Pure water, WWTP effluent	LC-LTQ Orbitrap-MS, NMR	Synergi Hydro C18 (4.0 x 250 mm, 4.0 µm)	MeOH/H_2O	NA	[11]
ACV-TP1	Ozonated TP					NA	[11]
1*H*-benzotriazole (BZT)	Anticorrosive	Pure water	LC-TOF-MS	XDB-C18 (4.6 x 50 mm, 1.8 µm)	ACN/H_2O	7.30	[12, 13]
BZT-TP1	Ozonated TP					3.71	[12]
BZT-TP2	Ozonated TP						[12]
BZT-TP3	Ozonated TP					1.85	[12]
BZT-TP4	Ozonated TP					1.34	[12]
BZT-TP5	Ozonated TP	Pure water, SW, WWTP	LC-Q-TOF-MS	HALO C18 (4.6 x 50 mm, 2.7 µm)	MeOH/H_2O		[13]
BZT-TP6	Ozonated TP	Pure water, SW, WWTP	LC-Q-TOF-MS	HALO C18 (4.6 x 50 mm, 2.7 µm)	MeOH/H_2O		[13]
BZT-TP7	Ozonated TP	Pure water, SW, WWTP	LC-Q-TOF-MS	HALO C18 (4.6 x 50 mm, 2.7 µm)	MeOH/H_2O		[13]
Methylindole (MLD)	Multi usages	Pure water	LC-TOF-MS	XDB-C18 (4.6 x 50 mm, 1.8 µm)	ACN/H_2O	14.60	[12]
MLD-TP1	Ozonated TP					13.75	[12]
MLD-TP2	Ozonated TP					9.23	[12]
MLD-TP3	Ozonated TP					3.77	[12]

Table 5.11: Suspect list including compounds, uses, lab study water matrices, analytical methods, analytical columns, and the used mobile phases obtained from literatures [1-32] (continued)

MLD-TP4	Ozonated TP					6.38	[12]
MLD-TP5	Ozonated TP					10.60	[12]
Imidacloprid (ICR)	Insecticide	Pure water	HPLC-DAD, HPLC-Q-Trap-MS	Kinetex C18 (2.1 x 150 mm, 1.7 µm)	ACN/H_2O	13.20	[14]
ICR-TP1	Ozonated TP						[14]
ICR-TP2	Ozonated TP						[14]
ICR-TP3	Ozonated TP					5.70	[14]
ICR-TP4	Ozonated TP						[14]
ICR-TP5	Ozonated TP						[14]
ICR-TP6	Ozonated TP					7.90	[14]
ICR-TP7	Ozonated TP					9.90	[14]
ICR-TP8	Ozonated TP						[14]
ICR-TP9	Ozonated TP						[14]
Propranolol (PRL)	Beta blocker	Pure water, WWTP effluent	HPLC-UV, LC–MS	Synergi 4u Hydro C18 (3.0 x 250 mm, 4.0 µm)	ACN/H_2O	48.5	[15]
PRL-TP1	Ozonated TP					32.50	[15]
PRL-TP2	Ozonated TP					26.90	[15]
PRL-TP3	Ozonated TP					41.80	[15]
PRL-TP4	Ozonated TP					20.10	[15]
Carbamazepine (CBZ)	Anticonvulsant	WWTP effluent	HPLC-MS/MS, UPLC-Q-TOF-MS/MS	Kinetex C18 (2.1 x 100 mm, 2.6 µm)	MeOH/H_2O	NA	[16]
CBZ-TP1	Ozonated TP					2.84	[16]
CBZ-TP2	Ozonated TP					2.20	[16]
CBZ-TP3	Ozonated TP					2.75	[16]
CBZ-TP4	Ozonated TP					2.02	[16]
CBZ-TP5	Ozonated TP					2.73	[16]
CBZ-TP6	Ozonated TP					2.47	[16]
CBZ-TP7	Ozonated TP					2.51	[16]
CBZ-TP8	Ozonated TP					2.88	[16]

Table 5.11: Suspect list including compounds, uses, lab study water matrices, analytical methods, analytical columns, and the used mobile phases obtained from literatures [1-32] (continued)

CBZ-TP9	Ozonated TP					3.11	[16]
CBZ-TP10	Ozonated TP					3.44	[16]
CBZ-TP11	Ozonated TP					0.90	[16]
CBZ-TP12	Ozonated TP						[16]
CBZ-TP13	Ozonated TP					1.51	[16]
CBZ-TP14	Ozonated TP					1.95	[16]
CBZ-TP15	Ozonated TP					2.65	[16]
Triclosan (TCS)	Antibacterial & antifungal	Pure water, WWTP	HPLC-MS/MS, GC-MS	Synergi 4u Polar C18 (2.0 x 150 mm, 4.0 µm)	MeOH/H_2O	30.78	[17]
TCS-TP1	Ozonated TP					24.76	[17]
TCS-TP2	Ozonated TP						[17]
TCS-TP3	Ozonated TP					27.56	[17]
TCS-TP4	Ozonated TP						[17]
Aminopyrine (AMP)	Analgesic	Purw water, SW	UPLC-Q-TOF-MS	Acquity C18 (2.1 x 50 mm, 1.7 µm)	ACN/H_2O	3.92	[18]
AMP-TP1	Ozonated TP						[18]
AMP-TP2	Ozonated TP						[18]
AMP-TP3	Ozonated TP						[18]
AMP-TP4	Ozonated TP						[18]
AMP-TP5	Ozonated TP						[18]
AMP-TP6	Ozonated TP					1.42	[18]
AMP-TP7	Ozonated TP					3.89	[18]
AMP-TP8	Ozonated TP					5.24	[18]
AMP-TP9	Ozonated TP					6.97	[18]
AMP-TP10	Ozonated TP						[18]
AMP-TP11	Ozonated TP					7.17	[18]
AMP-TP12	Ozonated TP					4.94	[18]
AMP-TP13	Ozonated TP					1.84	[18]

Table 5.11: Suspect list including compounds, uses, lab study water matrices, analytical methods, analytical columns, and the used mobile phases obtained from literatures [1-32] (continued)

Clarithromycin (CMC)	Antibiotic	Pure water, WWTP	HPLC-MS/MS, NMR	LUNA C8 (2.0 x 20 mm, 5.0 µm)	MeOH/H_2O	1.66	[19]
CMC-TP1	Ozonated TP					NA	[19]
CMC-TP2	Ozonated TP						[19]
Atenolol (ATL)	Beta blocker	Pure water	LC-Q-TOF-MS	SB-C18 (2.1 x 100 mm, 1.8 µm)	ACN/H_2O	2.365	[20]
ATL-TP1	Ozonated TP					1.463	[20]
ATL-TP2	Ozonated TP					1.108	[20]
ATL-TP3	Ozonated TP					3.816	[20]
ATL-TP4	Ozonated TP					1.279	[20]
ATL-TP5	Ozonated TP						[20]
ATL-TP6	Ozonated TP					1.827	[20]
ATL-TP7	Ozonated TP						[20]
ATL-TP8	Ozonated TP					1.270	[20]
ATL-TP9	Ozonated TP					1.204	[20]
17β-Estradiol (ESD)	Steroid hormone	Pure water, DWTP	GC–MS			NA	[21]
ESD-TP1	Ozonated TP					NA	[21]
ESD-TP2	Ozonated TP					NA	[21]
Diclofenac (DFC)	Analgesic	Pure water	LC-DAD, LC-Q-Trap-MS, NMR, FT-IR	Nucleodur C18 (3.0 x 250 mm)	ACN/H_2O	40.40	[22]
DFC-TP1	Ozonated TP					19.10	[22]
DFC-TP2	Ozonated TP					18.30	[22]
Metoprolol (MPL)	Beta blocker	Pure water	LC-Q-TOF-MS	SB-C18 (2.1 x 100 mm, 1.8 µm)	ACN/H_2O	6.328	[23]
MPL-TP1	Ozonated TP					0.981	[23]
MPL-TP2	Ozonated TP					1.072	[23]
MPL-TP3	Ozonated TP					5.058	[23]
MPL-TP4	Ozonated TP					3.110	[23]
MPL-TP5	Ozonated TP					3.182	[23]
MPL-TP6	Ozonated TP						[23]

Table 5.11: Suspect list including compounds, uses, lab study water matrices, analytical methods, analytical columns, and the used mobile phases obtained from literatures [1-32] (continued)

MPL-TP7	Ozonated TP						[23]
Sulfamethoxazole (SMZ)	Antibiotic	Pure water	LC-Q-TOF-MS	XDB-C18 (4.6 x 50 mm, 1.8 µm)	ACN/H_2O	NA	[24]
SMZ-TP1	Ozonated TP					NA	[24]
SMZ-TP2	Ozonated TP					NA	[24]
SMZ-TP3	Ozonated TP					NA	[24]
SMZ-TP4	Ozonated TP						[24]
Ciprofloxacin (CFX)	Antibiotic	Pure water, WW effluent	LC-Q-Trap-MS	Inertsil ODS-3 C18 (2.0 x150 mm, 5.0 µm)	MeOH/H_2O	24.77	[25]
CFX-TP1	Ozonated TP					22.22	[25]
CFX-TP2	Ozonated TP					35.14	[25]
CFX-TP3	Ozonated TP					31.70	[25]
CFX-TP4	Ozonated TP					30.40	[25]
CFX-TP5	Ozonated TP					30.04	[25]
CFX-TP6	Ozonated TP					34.90	[25]
CFX-TP7	Ozonated TP					28.20	[25]
CFX-TP8	Ozonated TP						[25]
CFX-TP9	Ozonated TP					36.97	[25]
CFX-TP10	Ozonated TP						[25]
CFX-TP11	Ozonated TP						[25]
CFX-TP12	Ozonated TP						[25]
CFX-TP13	Ozonated TP						[25]
CFX-TP14	Ozonated TP					20.69	[25]
CFX-TP15	Ozonated TP					18.47	[25]
CFX-TP16	Ozonated TP					17.18	[25]
Norfloxacin (NFX)	Antibacterial	Pure water, WW effluent	LC-Q-Trap-MS/MS	Inertsil ODS-3 C18 (2.0 x 150 mm, 5.0 µm)	MeOH/H_2O	23.91	[25]
NFX-TP1	Ozonated TP					21.16	[25]
NFX-TP2	Ozonated TP					34.12	[25]

Table 5.11: Suspect list including compounds, uses, lab study water matrices, analytical methods, analytical columns, and the used mobile phases obtained from literatures [1-32] (continued)

NFX-TP3	Ozonated TP					30.42	[25]
NFX-TP4	Ozonated TP					29.22	[25]
NFX-TP5	Ozonated TP						[25]
NFX-TP6	Ozonated TP						[25]
NFX-TP7	Ozonated TP					35.83	[25]
NFX-TP8	Ozonated TP						[25]
NFX-TP9	Ozonated TP						[25]
NFX-TP10	Ozonated TP					16.75	[25]
NFX-TP11	Ozonated TP					7.84	[25]
NFX-TP12	Ozonated TP					11.61	[25]
NFX-TP13	Ozonated TP					19.57	[25]
NFX-TP14	Ozonated TP					17.41	[25]
NFX-TP15	Ozonated TP					16.02	[25]
Paracetamol (PCM)	Analgesic	Pure water	GC–MS, NMR			NA	[26]
PCM-TP1	Ozonated TP					NA	[26]
Acesulfame (ACF)	Sweetener	Pure water, DWTP, Tap water, WWTP	LC-Q-TOF-MS, NMR, IC-ICP/MS	Synergie Hydro C18 (3.0 x 250 mm, 4.0 µm)	MeOH/H_2O	NA	[27]
ACF-TP1	Ozonated TP					NA	[27]
Cephalexin (CPX)	Antibiotic	WW effluent	HPLC-UV, LC-MS/MS, NMR, FT-IR	Nucleosil C18 (2.0 x 250 mm, 5.0 µm)	ACN/H_2O	39.50	[28]
CPX-TP1	Ozonated TP					22.00	[28]
CPX-TP2	Ozonated TP					23.50	[28]
CPX-TP3	Ozonated TP					32.50	[28]
Penicillin G (PG)	Antibacterial	WW effluent	HPLC-UV, LC-MS/MS, NMR, FT-IR	Discovery Amide (3.0 x 250 mm, 5.0 µm)	ACN/H_2O	12.50	[28]
PG-TP1	Ozonated TP					6.80	[28]
Progesterone (PGT)	Steroid hormone	Pure water	LC-Ion Trap-MS	Uptispher HDO C18 (3.0 x 250 mm, 5.0 µm)	MeOH/H_2O	51.11	[29]
PGT-TP1	Ozonated TP					7.43	[29]
PGT-TP2	Ozonated TP					37.58	[29]

Table 5.11: Suspect list including compounds, uses, lab study water matrices, analytical methods, analytical columns, and the used mobile phases obtained from literatures [1-32] (continued)

Tramadol (TMD)	Analgesic	Pure water	HPLC-UV/FLD, LC-Q-LIT-MS, LTQ-FT-MS, GC-MS	Synergie Hydro C18 (10.0 x 250 mm, 4.0 µm)	ACN/H_2O	NA	[30]
TMD-TP1	Ozonated TP					NA	[30]
TMD-TP2	Ozonated TP					NA	[30]
TMD-TP3	Ozonated TP					NA	[30]
TMD-TP4	Ozonated TP					NA	[30]
TMD-TP5	Ozonated TP					NA	[30]
TMD-TP6	Ozonated TP					NA	[30]
TMD-TP7	Ozonated TP						[30]
Venlafaxine (VFX)	Antidepressant	WW effluent	HPLC-MS/MS, GC-MS	ACE-C18 (2.1 x 250 mm)	MeOH/H_2O	NA	[31]
VFX-TP1	Ozonated TP					NA	[31]
Bezafibrate (BZR)	Lipid regulator	Pure water	HPLC-MS	Synergy Polar 4 C18	ACN/H_2O	9.80	[32]
BZR-TP1	Ozonated TP					3.20	[32]
BZR-TP2	Ozonated TP						[32]
BZR-TP3	Ozonated TP					6.40	[32]
BZR-TP4	Ozonated TP					8.70	[32]

ACN: Acetonitrile; DAD: Diode array detector; DWTP: Drinking water treatment plant; FLD: Fluorescence detector; FT: Fourier transform; GC: Gas chromatography; H_2O: Water; HPLC: High performance liquid chromatography; IC: Ion chromatography; ICP: Inductively coupled plasma; IR: Infrared; LC: Liquid chromatography; LIT: Linear ion trap; LTQ: Linear trap quadrupole; MeOH: Methanol; MS: Mass spectrometry; MS/MS: Tandem mass spectrometry; NA: Not available; NMR: Nuclear magnetic resonance; Q: Quadrupole; SW: Surface water; TLC: Thin layer chromatography; TOF: Time-of-flight; TP: Transformation product; UPLC: Ultra-performance liquid chromatography; UV:Ultraviolet; WW: Wastewater; WWTP: Wastewater treatment plant

Table 5.12: LC-Q-TOF-MS data obtained for the detected compounds using molecular-feature extraction database search

Compound	Formula	Exact mass	Polarity	Formula-2	Calculated mass	Measured mass	Mass error (ppm)	RT (min) [Experimental]
Bisphenol A (BPA)	$C_{15}H_{16}O_2$	228.1150	$[M+H]^+$	$C_{15}H_{17}O_2$	229.1229	229.1232	1.31	32.94
BPA-TP1	$C_{15}H_{16}O_5$	276.0998	$[M-H]^-$	$C_{15}H_{15}O_5$	275.0919	275.0925	2.18	7.12
BPA-TP2	$C_9H_{12}O_2$	152.0837	$[M-H]^-$	$C_9H_{11}O_2$	151.0759	151.0762	1.99	20.21
BPA-TP3	$C_{15}H_{16}O_3$	244.1099	$[M+H]^+$	$C_{15}H_{17}O_3$	245.1178	245.1172	-2.45	30.71
BPA-TP4	$C_{19}H_{18}O_5$	326.1154						
BPA-TP5	$C_{28}H_{28}O_6$	460.1886						
Caffeine (CAFF)	$C_8H_{10}N_4O_2$	194.0804	$[M-H]^-$	$C_8H_9N_4O_2$	193.0726	193.0727	0.52	31.10
CAFF-TP1	$C_8H_{10}N_4O_5$	242.0651	$[M+H]^+$	$C_8H_{11}N_4O_5$	243.0729	243.0733	1.65	25.47
CAFF-TP2	$C_8H_{12}N_4O_4$	228.0859	$[M+H]^+$	$C_8H_{13}N_4O_4$	229.0937	229.0931	-2.62	24.63
CAFF-TP3	$C_7H_{10}N_4O_3$	198.0753						
CAFF-TP4	$C_5H_8N_2O_3$	144.0535	$[M+H]^+$	$C_5H_9N_2O_3$	145.0613	145.0615	1.38	16.57
CAFF-TP5	$C_6H_9N_3O_4$	187.0593	$[M+H]^+$	$C_6H_{10}N_3O_4$	188.0671	188.0674	1.60	20.71
CAFF-TP6	$C_8H_{10}N_4O_4$	226.0702	$[M+H]^+$	$C_8H_{11}N_4O_4$	227.0780	227.0787	3.08	16.79
Estrone sulfate (EST-S)	$C_{18}H_{22}SO_5$	350.1188	$[M+H]^+$	$C_{18}H_{23}O_5S$	351.1266	351.1263	-0.85	43.35
EST-S-TP1	$C_{18}H_{22}SO_7$	382.1086	$[M-H]^-$	$C_{18}H_{21}SO_7$	381.1008	381.1012	1.05	29.10
EST-S-TP2	$C_{18}H_{18}SO_8$	394.0722	$[M-H]^-$	$C_{18}H_{17}SO_8$	393.0644	393.0651	1.78	29.57
EST-S-TP3	$C_{18}H_{20}SO_7$	380.0930	$[M-H]^-$	$C_{18}H_{19}SO_7$	379.0851	379.0848	-0.79	29.67
EST-S-TP4	$C_{18}H_{22}SO_6$	366.1137	$[M-H]^-$	$C_{18}H_{21}SO_6$	365.1059	365.1063	1.10	33.69
EST-S-TP5	$C_{18}H_{20}SO_8$	396.0879	$[M-H]^-$	$C_{18}H_{19}SO_8$	395.0801	395.0807	1.52	33.86
EST-S-TP6	$C_{18}H_{22}SO_8$	398.1035	$[M-H]^-$	$C_{18}H_{21}SO_8$	397.0957	397.0968	2.77	33.99
EST-S-TP7	$C_{18}H_{24}SO_8$	400.1192	$[M+H]^+$	$C_{18}H_{25}SO_8$	401.1270	401.1279	2.24	34.49
EST-S-TP8	$C_{18}H_{18}SO_7$	378.0773	$[M-H]^-$	$C_{18}H_{17}SO_7$	377.0695	377.0690	-1.33	37.81

Table 5.12: LC-Q-TOF-MS data obtained for the detected compounds using molecular-feature extraction database search (continued)

EST-S-TP9	$C_{18}H_{20}SO_6$	364.0981	$[M+H]^+$	$C_{18}H_{21}SO_6$	365.1059	365.1052	-1.92	40.33
Trimethoprim (TMP)	$C_{14}H_{18}N_4O_3$	290.1379	$[M+H]^+$	$C_{14}H_{19}N_4O_3$	291.1457	291.1455	-0.69	40.05
TMP-TP1	$C_{13}H_{18}N_4O_4$	294.1328	$[M+H]^+$	$C_{13}H_{19}N_4O_4$	295.1406	295.1412	2.03	21.83
TMP-TP2	$C_{14}H_{18}N_4O_5$	322.1277						
TMP-TP3	$C_{14}H_{20}N_4O_5$	324.1434	$[M+H]^+$	$C_{14}H_{21}N_4O_5$	325.1512	325.1518	1.85	26.28
TMP-TP4	$C_{14}H_{18}N_4O_6$	338.1226						
TMP-TP5	$C_{13}H_{16}N_4O_3$	276.1222	$[M+H]^+$	$C_{13}H_{17}N_4O_3$	277.1301	277.1309	2.89	26.06
TMP-TP6	$C_{13}H_{15}N_3O_4$	277.1063						
TMP-TP7	$C_{12}H_{16}N_4O_4$	280.1172	$[M+H]^+$	$C_{12}H_{17}N_4O_4$	281.1250	281.1259	3.20	15.88
TMP-TP8	$C_{14}H_{18}N_2O_6$	310.1165	$[M+H]^+$	$C_{14}H_{19}N_2O_6$	311.1243	311.1239	-1.29	16.90
TMP-TP9	$C_{14}H_{18}N_4O_7$	354.1175						
TMP-TP10	$C_{14}H_{20}N_4O_7$	356.1332	$[M+H]^+$	$C_{14}H_{21}N_4O_7$	357.1410	357.1419	2.52	17.85
TMP-TP11	$C_{11}H_{13}N_3O_3$	235.0957	$[M+H]^+$	$C_{11}H_{14}N_3O_3$	236.1035	236.1031	-1.69	32.18
TMP-TP12	$C_{14}H_{18}N_4O_4$	306.1328	$[M+H]^+$	$C_{14}H_{19}N_4O_4$	307.1406	307.1411	1.63	24.88
TMP-TP13	$C_{11}H_{16}N_4O_7$	316.1019						
TMP-TP14	$C_5H_6N_4O$	138.0542	$[M+H]^+$	$C_5H_7N_4O$	139.0620	139.0624	2.88	20.88
TMP-TP15	$C_5H_8N_4O$	140.0698	$[M+H]^+$	$C_5H_9N_4O$	141.0776	141.0773	-2.13	9.88
Roxithromycin (ROX)	$C_{41}H_{76}N_2O_{15}$	836.5246	$[M+H]^+$	$C_{41}H_{77}N_2O_{15}$	837.5324	837.5331	0.84	34.55
ROX-TP1	$C_{33}H_{62}N_2O_{13}$	694.4252	$[M+H]^+$	$C_{33}H_{63}N_2O_{13}$	695.433	695.4339	1.29	24.73
ROX-TP2	$C_{40}H_{74}N_2O_{15}$	822.5089	$[M+H]^+$	$C_{40}H_{75}N_2O_{15}$	823.5167	823.5155	-1.46	31.61
ROX-TP3	$C_{40}H_{74}N_2O_{16}$	838.5038	$[M+H]^+$	$C_{40}H_{75}N_2O_{16}$	839.5117	839.5111	-0.71	35.56
ROX-TP4	$C_{41}H_{74}N_2O_{16}$	850.5038	$[M+H]^+$	$C_{41}H_{75}N_2O_{16}$	851.5117	851.5129	1.41	38.68
ROX-TP5	$C_{41}H_{76}N_2O_{16}$	852.5195	$[M+H]^+$	$C_{41}H_{77}N_2O_{16}$	853.5273	853.5289	1.87	34.92
Methylbenzotriazole (MBZ)	$C_7H_7N_3$	133.0640	$[M+H]^+$	$C_7H_8N_3$	134.0718	134.0715	-2.24	23.32

Table 5.12: LC-Q-TOF-MS data obtained for the detected compounds using molecular-feature extraction database search (continued)

MBZ-TP1	$C_5H_5N_3O_2$	139.0382	$[M-H]^-$	$C_5H_4N_3O_2$	138.0304	138.0307	2.17	16.84
MBZ-TP2	$C_7H_5N_3O$	147.0433	$[M-H]^-$	$C_7H_4N_3O$	146.0354	146.0358	2.74	18.77
MBZ-TP3	$C_7H_7N_3O$	149.0589	$[M+H]^+$	$C_7H_8N_3O$	150.0667	150.0666	-0.67	18.48
MBZ-TP4	$C_7H_5N_3O_2$	163.0382	$[M+H]^+$	$C_7H_6N_3O_2$	164.0460	164.0455	-3.05	17.25
MBZ-TP5	$C_7H_7N_3O_2$	165.0538	$[M+H]^+$	$C_7H_8N_3O_2$	166.0617	166.0621	2.41	17.93
MBZ-TP6	$C_7H_5N_3O_3$	179.0331						
MBZ-TP7	$C_7H_7N_3O_3$	181.0487						
MBZ-TP8	$C_7H_5N_3O_4$	195.0280						
MBZ-TP9	$C_7H_7N_3O_4$	197.0437						
MBZ-TP10	$C_7H_5N_3O_5$	211.0229						
MBZ-TP11	$C_7H_5N_3O_6$	227.0178						
Imazalil (IMZ)	$C_{14}H_{14}Cl_2N_2O$	296.0483	$[M+H]^+$	$C_{14}H_{15}Cl_2N_2O$	297.0561	297.0558	-1.01	45.99
IMZ-TP1	$C_{13}H_{12}Cl_2N_2O_2$	298.0276	$[M+H]^+$	$C_{13}H_{13}Cl_2N_2O_2$	299.0354	299.0363	3.01	25.21
IMZ-TP2	$C_{11}H_{12}Cl_2N_2O_2$	274.0276						
IMZ-TP3	$C_{11}H_{11}Cl_2NO_3$	275.0116	$[M+H]^+$	$C_{11}H_{12}Cl_2NO_3$	276.0194	276.0185	-3.26	28.61
IMZ-TP4	$C_{13}H_{15}Cl_2NO_5$	335.0327	$[M+H]^+$	$C_{13}H_{14}Cl_2NO_5$	334.0249	334.0243	-1.80	26.33
Ketoprofen (KPR)	$C_{16}H_{14}O_3$	254.0943	$[M+H]^+$	$C_{16}H_{15}O_3$	255.1021	255.1026	1.96	32.35
KPR-TP1	$C_{15}H_{14}O$	210.1045						
KPR-TP2	$C_{15}H_{14}O_2$	226.0994	$[M+H]^+$	$C_{15}H_{15}O_2$	227.1072	227.1078	2.64	30.30
KPR-TP3	$C_{15}H_{14}O_3$	242.0943	$[M-H]^-$	$C_{15}H_{13}O_3$	241.0865	241.0871	2.49	36.33
KPR-TP4	$C_{15}H_{12}O_2$	224.0837	$[M+H]^+$	$C_{15}H_{13}O_2$	225.0916	225.0911	-2.22	43.66
Levofloxacin (LVX)	$C_{18}H_{20}FN_3O_4$	361.1438	$[M+H]^+$	$C_{18}H_{21}FN_3O_4$	362.1516	362.1522	1.66	19.70
LVX-TP1	$C_{17}H_{20}FN_3O_6$	381.1336						
LVX-TP2	$C_{16}H_{20}FN_3O_5$	353.1387	$[M+H]^+$	$C_{16}H_{21}FN_3O_5$	354.1465	354.1469	1.13	16.69

Table 5.12: LC-Q-TOF-MS data obtained for the detected compounds using molecular-feature extraction database search (continued)

LVX-TP3	$C_{17}H_{18}FN_3O_4$	347.1281	$[M+H]^+$	$C_{17}H_{19}FN_3O_4$	348.1360	348.1354	-1.72	24.33
LVX-TP4	$C_{16}H_{20}FN_3O_4$	337.1438	$[M+H]^+$	$C_{16}H_{21}FN_3O_4$	338.1516	338.1512	-1.18	12.18
LVX-TP5	$C_{16}H_{18}FN_3O_4$	335.1281						
LVX-TP6	$C_{18}H_{20}FN_3O_5$	377.1387	$[M+H]^+$	$C_{18}H_{21}FN_3O_5$	378.1465	378.1473	2.12	27.19
LVX-TP7	$C_{13}H_{11}FN_2O_4$	278.0703	$[M+H]^+$	$C_{13}H_{12}FN_2O_4$	279.0781	279.0779	-0.72	38.15
Chlorophene (CLP)	$C_{13}H_{11}ClO$	218.0498	$[M-H]^-$	$C_{13}H_{10}ClO$	217.0420	217.0423	1.38	42.84
CLP-TP1	$C_7H_5ClO_3$	171.9927	$[M-H]^-$	$C_7H_4ClO_3$	170.9849	170.9852	1.75	33.15
CLP-TP2	$C_{13}H_{11}ClO_2$	234.0448	$[M+H]^+$	$C_{13}H_{12}ClO_2$	235.0526	235.0532	2.55	35.95
CLP-TP3	$C_{13}H_{10}O_4$	230.0579						
CLP-TP4	$C_{12}H_7ClO_{10}$	345.9728	$[M-H]^-$	$C_{12}H_6ClO_{10}$	344.9649	344.9656	2.03	22.77
CLP-TP5	$C_{12}H_{12}O_3$	204.0786	$[M+H]^+$	$C_{12}H_{13}O_3$	205.0865	205.0863	-0.98	25.10
CLP-TP6	$C_{11}H_{10}O_4$	206.0579	$[M+H]^+$	$C_{11}H_{11}O_4$	207.0657	207.0660	1.45	22.42
CLP-TP7	$C_{12}H_8O_{10}$	312.0117						
CLP-TP8	$C_{12}H_{12}O_5$	236.0685	$[M+H]^+$	$C_{12}H_{13}O_5$	237.0763	237.0761	-0.84	20.91
CLP-TP9	$C_{11}H_{10}O_5$	222.0528	$[M-H]^-$	$C_{11}H_9O_5$	221.0450	221.0457	3.17	22.14
CLP-TP10	$C_9H_{12}O_{10}$	280.0430	$[M+H]^+$	$C_9H_{13}O_{10}$	281.0509	281.0504	-1.78	23.13
Acyclovir (ACV)	$C_8H_{11}N_5O_3$	225.0862	$[M-H]^-$	$C_8H_{10}N_5O_3$	224.0784	224.0789	2.23	34.64
ACV-TP	$C_8H_{13}N_5O_5$	259.0917	$[M+H]^+$	$C_8H_{14}N_5O_5$	260.0995	260.0987	-3.08	33.38
1*H*-benzotriazole (BZT)	$C_6H_5N_3$	119.0483	$[M+H]^+$	$C_6H_6N_3$	120.0562	120.0565	2.50	19.79
BZT-TP1	$C_6H_5N_3O$	135.0433	$[M+H]^+$	$C_6H_6N_3O$	136.0511	136.0509	-1.47	10.23
BZT-TP2	$C_6H_5N_3O_2$	151.0382						
BZT-TP3	$C_6H_5N_3O_3$	167.0331	$[M-H]^-$	$C_6H_4N_3O_3$	166.0253	166.0257	2.41	5.24
BZT-TP4	$C_6H_7N_3O_3$	169.0487	$[M+H]^+$	$C_6H_8N_3O_3$	170.0566	170.0562	-2.35	4.14
BZT-TP5	$C_4H_3N_3O_2$	125.0225						

Table 5.12: LC-Q-TOF-MS data obtained for the detected compounds using molecular-feature extraction database search (continued)

BZT-TP6	$C_4H_5N_3O_3$	143.0331						
BZT-TP7	$C_5H_5N_3O_3$	155.0331						
Methylindole (MLD)	C_9H_9N	131.0735	$[M+H]^+$	$C_9H_{10}N$	132.0813	132.0815	1.51	21.35
MLD-TP1	C_9H_9NO	147.0684	$[M-H]^-$	C_9H_8NO	146.0606	146.0602	-2.74	20.95
MLD-TP2	$C_9H_9NO_2$	163.0633	$[M+H]^+$	$C_9H_{10}NO_2$	164.0712	164.0714	1.22	14.04
MLD-TP3	$C_9H_{11}NO_2$	165.0790	$[M+H]^+$	$C_9H_{12}NO_2$	166.0868	166.0863	-3.01	7.43
MLD-TP4	$C_9H_9NO_3$	179.0582	$[M+H]^+$	$C_9H_{10}NO_3$	180.0661	180.0665	2.22	10.87
MLD-TP5	C_8H_9NO	135.0684	$[M+H]^+$	$C_8H_{10}NO$	136.0762	136.0758	-2.94	18.80
Imidacloprid (ICR)	$C_9H_{10}ClN_5O_2$	255.0523	$[M+H]^+$	$C_9H_{11}ClN_5O_2$	256.0601	256.0599	-0.78	31.30
ICR-TP1	C_6H_4ClNO	140.9981						
ICR-TP2	$C_7H_8ClN_5O_2$	229.0367						
ICR-TP3	$C_9H_{10}ClN_5O_4$	287.0421	$[M+H]^+$	$C_9H_{11}ClN_5O_4$	288.0500	288.0508	2.78	12.97
ICR-TP4	$C_6H_4ClNO_2$	156.9931						
ICR-TP5	$C_9H_{10}ClN_5O_3$	271.0472						
ICR-TP6	$C_9H_6ClN_3O_3$	239.0098	$[M-H]^-$	$C_9H_5ClN_3O_3$	238.0019	238.0014	-2.10	22.64
ICR-TP7	$C_9H_8ClN_5O_3$	269.0316	$[M+H]^+$	$C_9H_9ClN_5O_3$	270.0394	270.0391	-1.11	23.44
ICR-TP8	$C_9H_8ClN_5O_4$	285.0265						
ICR-TP9	$C_6H_7ClN_2$	142.0298						
Propranolol (PRL)	$C_{16}H_{21}NO_2$	259.1572	$[M+H]^+$	$C_{16}H_{22}NO_2$	260.1651	260.1647	-1.54	38.50
PRL-TP1	$C_{14}H_{19}NO_4$	265.1314	$[M+H]^+$	$C_{14}H_{20}NO_4$	266.1392	266.1393	0.38	28.15
PRL-TP2	$C_{14}H_{19}NO_5$	281.1263	$[M+H]^+$	$C_{14}H_{20}NO_5$	282.1341	282.1345	1.42	20.64
PRL-TP3	$C_{16}H_{21}NO_4$	291.1471	$[M+H]^+$	$C_{16}H_{22}NO_4$	292.1549	292.1555	2.05	34.26
PRL-TP4	$C_{16}H_{21}NO_5$	307.1420	$[M-H]^-$	$C_{16}H_{20}NO_5$	306.1341	306.1352	3.59	17.54
Carbamazepine (CBZ)	$C_{15}H_{12}N_2O$	236.0950	$[M+H]^+$	$C_{15}H_{13}N_2O$	237.1028	237.1025	-1.27	32.50

Table 5.12: LC-Q-TOF-MS data obtained for the detected compounds using molecular-feature extraction database search (continued)

CBZ-TP1	$C_{14}H_9NO_2$	223.0633	$[M+H]^+$	$C_{14}H_{10}NO_2$	224.0712	224.0716	1.79	28.90
CBZ-TP2	$C_{15}H_{14}N_2O_3$	270.1004	$[M+H]^+$	$C_{15}H_{15}N_2O_3$	271.1083	271.1085	0.74	25.31
CBZ-TP3	$C_{15}H_{10}N_2O_3$	266.0691	$[M+H]^+$	$C_{15}H_{11}N_2O_3$	267.0770	267.0763	-2.62	28.21
CBZ-TP4	$C_{15}H_{10}N_2O_4$	282.0641	$[M+H]^+$	$C_{15}H_{11}N_2O_4$	283.0719	283.0715	-1.41	24.56
CBZ-TP5	$C_{15}H_{12}N_2O_2$	252.0899	$[M+H]^+$	$C_{15}H_{13}N_2O_2$	253.0977	253.0976	-0.40	27.38
CBZ-TP6	$C_{15}H_{12}N_2O_3$	268.0848	$[M+H]^+$	$C_{15}H_{13}N_2O_3$	269.0926	269.0923	-1.11	26.38
CBZ-TP7	$C_{15}H_{10}N_2O_2$	250.0742	$[M+H]^+$	$C_{15}H_{11}N_2O_2$	251.0821	251.0823	0.80	26.65
CBZ-TP8	$C_{13}H_9NO$	195.0684	$[M+H]^+$	$C_{13}H_{10}NO$	196.0762	196.0759	-1.53	29.29
CBZ-TP9	$C_{14}H_{11}NO_4$	257.0688	$[M+H]^+$	$C_{14}H_{12}NO_4$	258.0766	258.0758	-3.10	30.20
CBZ-TP10	$C_{14}H_9NO$	207.0684	$[M+H]^+$	$C_{14}H_{10}NO$	208.0762	208.0764	0.96	30.99
CBZ-TP11	$C_{13}H_{12}N_2O_6$	292.0695	$[M+H]^+$	$C_{13}H_{11}N_2O_6$	291.0617	291.0611	-2.06	17.98
CBZ-TP12	$C_{13}H_{10}N_2O_6$	290.0539						
CBZ-TP13	$C_{12}H_8N_2O_4$	244.0484	$[M+H]^+$	$C_{12}H_9N_2O_4$	245.0562	245.0554	-3.26	21.41
CBZ-TP14	$C_{14}H_{10}N_2O_4$	270.0641	$[M+H]^+$	$C_{14}H_{11}N_2O_4$	271.0719	271.0726	2.58	24.12
CBZ-TP15	$C_{12}H_7NO_3$	213.0426	$[M+H]^+$	$C_{12}H_8NO_3$	214.0504	214.0501	-1.40	26.92
Triclosan (TCS)	$C_{12}H_7Cl_3O_2$	287.9512	$[M-H]^-$	$C_{12}H_6Cl_3O_2$	286.9433	286.9437	1.39	41.13
TCS-TP1	$C_6H_4Cl_2O$	161.9639	$[M-H]^-$	$C_6H_3Cl_2O$	160.9561	160.9566	3.11	30.56
TCS-TP2	$C_6H_5ClO_2$	143.9978						
TCS-TP3	$C_{12}H_7Cl_3O_3$	303.9461	$[M-H]^-$	$C_{12}H_6Cl_3O_3$	302.9383	302.9377	-1.98	35.35
TCS-TP4	$C_{12}H_7Cl_3O_4$	319.9410						
Aminopyrine (AMP)	$C_{13}H_{17}N_3O$	231.1372	$[M+H]^+$	$C_{13}H_{18}N_3O$	232.1450	232.1454	1.72	15.02
AMP-TP1	$C_{13}H_{17}N_3O_3$	263.1270						
AMP-TP2	$C_{12}H_{15}N_3O_3$	249.1113						
AMP-TP3	$C_{11}H_{15}N_3O_2$	221.1164						

Table 5.12: LC-Q-TOF-MS data obtained for the detected compounds using molecular-feature extraction database search (continued)

AMP-TP4	$C_9H_{12}N_2O$	164.0950						
AMP-TP5	$C_{11}H_{12}N_2O_4$	236.0797						
AMP-TP6	$C_9H_{12}N_2O_2$	180.0899	$[M+H]^+$	$C_9H_{13}N_2O_2$	181.0977	181.0979	1.10	6.54
AMP-TP7	$C_{12}H_{15}N_3O$	217.1215	$[M+H]^+$	$C_{12}H_{16}N_3O$	218.1293	218.1289	-1.83	14.11
AMP-TP8	$C_{11}H_{13}N_3O$	203.1059	$[M+H]^+$	$C_{11}H_{14}N_3O$	204.1137	204.1132	-2.45	22.27
AMP-TP9	$C_{11}H_{12}N_2O_2$	204.0899	$[M+H]^+$	$C_{11}H_{13}N_2O_2$	205.0977	205.098	1.46	28.45
AMP-TP10	$C_{13}H_{17}N_3O_2$	247.1321						
AMP-TP11	$C_{12}H_{13}N_3O_2$	231.1008	$[M+H]^+$	$C_{12}H_{14}N_3O_2$	232.1086	232.1084	-0.86	29.41
AMP-TP12	$C_{13}H_{17}N_3O_4$	279.1219	$[M+H]^+$	$C_{13}H_{18}N_3O_4$	280.1297	280.1301	1.43	16.86
AMP-TP13	$C_7H_{13}N_3O$	155.1059	$[M+H]^+$	$C_7H_{14}N_3O$	156.1137	156.1133	-2.56	8.75
Clarithromycin (CMC)	$C_{38}H_{69}NO_{13}$	747.4769	$[M+H]^+$	$C_{38}H_{70}NO_{13}$	748.4847	748.4842	-0.67	25.79
CMC-TP1	$C_{38}H_{69}NO_{14}$	763.4718	$[M+H]^+$	$C_{38}H_{70}NO_{14}$	764.4796	764.4789	-0.92	23.08
CMC-TP2	$C_{37}H_{67}NO_{13}$	733.4612						
Atenolol (ATL)	$C_{14}H_{22}N_2O_3$	266.1630	$[M+H]^+$	$C_{14}H_{23}N_2O_3$	267.1709	267.1704	-1.87	41.92
ATL-TP1	$C_8H_{15}NO_5$	205.0950	$[M+H]^+$	$C_8H_{16}NO_5$	206.1028	206.1034	2.91	26.78
ATL-TP2	$C_{11}H_{16}N_2O_3$	224.1161	$[M+H]^+$	$C_{11}H_{17}N_2O_3$	225.1239	225.1235	-1.78	15.65
ATL-TP3	$C_{13}H_{19}NO_3$	237.1365	$[M+H]^+$	$C_{13}H_{20}NO_3$	238.1443	238.1448	2.10	58.66
ATL-TP4	$C_{12}H_{20}N_2O_5$	272.1372	$[M+H]^+$	$C_{12}H_{21}N_2O_5$	273.1450	273.1457	2.56	26.18
ATL-TP5	$C_{14}H_{18}N_2O_4$	278.1267						
ATL-TP6	$C_{14}H_{20}N_2O_4$	280.1423	$[M+H]^+$	$C_{14}H_{21}N_2O_4$	281.1501	281.1503	0.71	31.99
ATL-TP7	$C_{14}H_{22}N_2O_4$	282.1580						
ATL-TP8	$C_{14}H_{22}N_2O_5$	298.1529	$[M+H]^+$	$C_{14}H_{23}N_2O_5$	299.1607	299.1613	2.01	25.38
ATL-TP9	$C_{14}H_{22}N_2O_6$	314.1478	$[M+H]^+$	$C_{14}H_{23}N_2O_6$	315.1556	315.1551	-1.59	24.41
17β-Estradiol (ESD)	$C_{18}H_{24}O_2$	272.1776	$[M+H]^+$	$C_{18}H_{25}O_2$	273.1855	273.1853	-0.73	43.46

Table 5.12: LC-Q-TOF-MS data obtained for the detected compounds using molecular-feature extraction database search (continued)

ESD-TP1	$C_{18}H_{24}O_3$	288.1725	$[M+H]^+$	$C_{18}H_{25}O_3$	289.1804	289.1797	-2.42	38.92
ESD-TP2	$C_{18}H_{30}O_2$	278.2246	$[M+H]^+$	$C_{18}H_{31}O_2$	279.2324	279.2318	-2.15	42.60
Diclofenac (DFC)	$C_{14}H_{11}Cl_2NO_2$	295.0167	$[M+H]^+$	$C_{14}H_{12}Cl_2NO_2$	296.0245	296.0242	-1.01	36.56
DFC-TP1	$C_{14}H_{11}Cl_2NO_3$	311.0116	$[M+H]^+$	$C_{14}H_{12}Cl_2NO_3$	312.0194	312.0194	0.00	18.62
DFC-TP2	$C_{14}H_9Cl_2NO_3$	308.9959	$[M+H]^+$	$C_{14}H_{10}Cl_2NO_3$	310.0038	310.0034	-1.29	16.39
Metoprolol (MPL)	$C_{15}H_{25}NO_3$	267.1834	$[M+H]^+$	$C_{15}H_{26}NO_3$	268.1913	268.1908	-1.86	25.58
MPL-TP1	$C_6H_{15}NO_2$	133.1103	$[M+H]^+$	$C_6H_{16}NO_2$	134.1181	134.1177	-2.98	4.76
MPL-TP2	$C_8H_{17}NO_5$	207.1107	$[M+H]^+$	$C_8H_{18}NO_5$	208.1185	208.1190	2.40	6.85
MPL-TP3	$C_{12}H_{19}NO_3$	225.1365	$[M+H]^+$	$C_{12}H_{20}NO_3$	226.1443	226.1447	1.77	21.06
MPL-TP4	$C_{13}H_{23}NO_5$	273.1576	$[M+H]^+$	$C_{13}H_{24}NO_5$	274.1654	274.1649	-1.82	11.75
MPL-TP5	$C_{15}H_{25}NO_4$	283.1784	$[M+H]^+$	$C_{15}H_{26}NO_4$	284.1862	284.1865	1.06	12.83
MPL-TP6	$C_{15}H_{25}NO_5$	299.1733						
MPL-TP7	$C_{15}H_{25}NO_7$	331.1631						
Sulfamethoxazole (SMZ)	$C_{10}H_{11}N_3O_3S$	253.0521	$[M+H]^+$	$C_{10}H_{12}N_3O_3S$	254.0599	254.0597	-0.79	30.83
SMZ-TP1	$C_4H_6N_2O$	98.0480	$[M+H]^+$	$C_4H_7N_2O$	99.0558	99.0560	2.02	20.33
SMZ-TP2	$C_{10}H_{13}N_3O_5S$	287.0576	$[M+H]^+$	$C_{10}H_{14}N_3O_5S$	288.0654	288.0659	1.74	21.95
SMZ-TP3	$C_{10}H_9N_3O_5S$	283.0263	$[M-H]^-$	$C_{10}H_7N_3O_5S$	281.0106	281.0111	1.78	24.98
SMZ-TP4	$C_{10}H_{11}N_3O_4S$	269.0470						
Ciprofloxacin (CFX)	$C_{17}H_{18}O_3N_3F$	331.1332	$[M+H]^+$	$C_{17}H_{19}O_3N_3F$	332.141	332.1409	-0.30	20.48
CFX-TP1	$C_{15}H_{16}O_3N_3F$	305.1176	$[M+H]^+$	$C_{15}H_{17}O_3N_3F$	306.1254	306.1256	0.65	18.92
CFX-TP2	$C_{13}H_{11}O_3N_2F$	262.0754	$[M+H]^+$	$C_{13}H_{12}O_3N_2F$	263.0832	263.0825	-2.66	29.18
CFX-TP3	$C_{17}H_{16}O_4N_3F$	345.1125	$[M+H]^+$	$C_{17}H_{17}O_4N_3F$	346.1203	346.1206	0.87	26.12
CFX-TP4	$C_{17}H_{14}O_5N_3F$	359.0917	$[M+H]^+$	$C_{17}H_{15}O_5N_3F$	360.0996	360.1005	2.50	23.71
CFX-TP5	$C_{17}H_{16}O_5N_3F$	361.1074	$[M+H]^+$	$C_{17}H_{17}O_5N_3F$	362.1152	362.1159	1.93	22.08

Table 5.12: LC-Q-TOF-MS data obtained for the detected compounds using molecular-feature extraction database search (continued)

CFX-TP6	$C_{14}H_{11}O_4N_2F$	290.0703	$[M+H]^+$	$C_{14}H_{12}O_4N_2F$	291.0781	291.0773	-2.75	27.75
CFX-TP7	$C_{16}H_{16}O_5N_3F$	349.1074	$[M+H]^+$	$C_{16}H_{17}O_5N_3F$	350.1152	350.1154	0.57	21.52
CFX-TP8	$C_{17}H_{18}O_5N_3F$	363.1230						
CFX-TP9	$C_{15}H_{14}O_4N_3F$	319.0968	$[M+H]^+$	$C_{15}H_{15}O_4N_3F$	320.1047	320.1041	-1.87	31.40
CFX-TP10	$C_{15}H_{14}O_5N_3F$	335.0917						
CFX-TP11	$C_{15}H_{18}O_3N_3F$	307.1332						
CFX-TP12	$C_{15}H_{16}O_5N_3F$	337.1074						
CFX-TP13	$C_{14}H_{16}O_4N_3F$	309.1125						
CFX-TP14	$C_{15}H_{16}O_2N_3F$	289.1227	$[M+H]^+$	$C_{15}H_{17}O_2N_3F$	290.1305	290.1299	-2.07	17.66
CFX-TP15	$C_{13}H_{16}O_3N_3F$	281.1176	$[M-H]^-$	$C_{13}H_{15}O_3N_3F$	280.1097	280.1103	2.14	17.49
CFX-TP16	$C_{13}H_{14}O_2N_3F$	263.1070	$[M+H]^+$	$C_{13}H_{15}O_2N_3F$	264.1148	264.1155	2.65	13.95
Norfloxacin (NFX)	$C_{16}H_{18}O_3N_3F$	319.1332	$[M+H]^+$	$C_{16}H_{19}O_3N_3F$	320.141	320.1413	0.94	17.02
NFX-TP1	$C_{14}H_{16}O_3N_3F$	293.1176	$[M+H]^+$	$C_{14}H_{17}O_3N_3F$	294.1254	294.1261	2.38	14.20
NFX-TP2	$C_{12}H_{11}O_3N_2F$	250.0754	$[M+H]^+$	$C_{12}H_{12}O_3N_2F$	251.0832	251.0836	1.59	24.49
NFX-TP3	$C_{16}H_{16}O_4N_3F$	333.1125	$[M+H]^+$	$C_{16}H_{17}O_4N_3F$	334.1203	334.1211	2.39	21.56
NFX-TP4	$C_{16}H_{14}O_5N_3F$	347.0917	$[M+H]^+$	$C_{16}H_{15}O_5N_3F$	348.0996	348.0999	0.86	19.95
NFX-TP5	$C_{15}H_{16}O_4N_3F$	321.1125						
NFX-TP6	$C_{16}H_{18}O_5N_3F$	351.1230						
NFX-TP7	$C_{14}H_{14}O_4N_3F$	307.0968	$[M+H]^+$	$C_{14}H_{15}O_4N_3F$	308.1047	308.1051	1.30	25.69
NFX-TP8	$C_{14}H_{14}O_5N_3F$	323.0917						
NFX-TP9	$C_{15}H_{18}O_4N_3F$	323.1281						
NFX-TP10	$C_{14}H_{18}O_3N_3F$	295.1332	$[M+H]^+$	$C_{14}H_{19}O_3N_3F$	296.1410	296.1419	3.04	12.69
NFX-TP11	$C_{14}H_{16}O_5N_3F$	325.1074	$[M+H]^+$	$C_{14}H_{17}O_5N_3F$	326.1152	326.1144	-2.45	6.92
NFX-TP12	$C_{13}H_{16}O_4N_3F$	297.1125	$[M+H]^+$	$C_{13}H_{17}O_4N_3F$	298.1203	298.1198	-1.68	9.45

Table 5.12: LC-Q-TOF-MS data obtained for the detected compounds using molecular-feature extraction database search (continued)

NFX-TP13	$C_{14}H_{16}O_2N_3F$	277.1227	$[M+H]^+$	$C_{14}H_{17}O_2N_3F$	278.1305	278.1313	2.88	13.49
NFX-TP14	$C_{12}H_{16}O_3N_3F$	269.1176	$[M-H]^-$	$C_{12}H_{15}O_3N_3F$	268.1097	268.1088	-3.36	13.31
NFX-TP15	$C_{12}H_{14}O_2N_3F$	251.1070	$[M+H]^+$	$C_{12}H_{15}O_2N_3F$	252.1148	252.1153	1.98	12.29
Paracetamol (PCM)	$C_8H_9NO_2$	151.0633	$[M+H]^+$	$C_8H_{10}NO_2$	152.0712	152.0709	-1.97	15.15
PCM-TP1	$C_8H_9NO_3$	167.0582	$[M+H]^+$	$C_8H_{10}NO_3$	168.0661	168.0659	-1.19	14.46
Acesulfame (ACF)	$C_4H_5NO_4S$	162.9939	$[M-H]^-$	$C_4H_4NO_4S$	161.9861	161.9864	1.85	23.97
ACF-TP1	$C_2H_4NO_6S$	169.9759	$[M-H]^-$	$C_2H_3NO_6S$	168.9681	168.9677	-2.37	14.72
Cephalexin (CPX)	$C_{16}H_{17}N_3O_4S$	347.0940	$[M+H]^+$	$C_{16}H_{18}N_3O_4S$	348.1018	348.1023	1.44	31.81
CPX-TP1	$C_{16}H_{17}N_3O_5S$	363.0889	$[M+H]^+$	$C_{16}H_{18}N_3O_5S$	364.0967	364.0956	-3.02	17.77
CPX-TP2	$C_{16}H_{19}N_3O_7S$	397.0944	$[M+H]^+$	$C_{16}H_{20}N_3O_7S$	398.1022	398.1028	1.51	19.41
CPX-TP3	$C_{16}H_{17}N_3O_6S$	379.0838	$[M+H]^+$	$C_{16}H_{18}N_3O_6S$	380.0916	380.0920	1.05	26.44
Penicillin G (PG)	$C_{16}H_{18}N_2O_4S$	334.0987	$[M+H]^+$	$C_{16}H_{19}N_2O_4S$	335.1066	335.1069	0.90	50.71
PG-TP1	$C_{16}H_{18}N_2O_5S$	350.0936	$[M+H]^+$	$C_{16}H_{19}N_2O_5S$	351.1015	351.1007	-2.28	26.24
Progesterone (PGT)	$C_{21}H_{30}O_2$	314.2246	$[M+H]^+$	$C_{21}H_{31}O_2$	315.2324	315.2330	1.90	43.86
PGT-TP1	$C_{20}H_{30}O_4$	334.2144	$[M-H]^-$	$C_{20}H_{29}O_4$	333.2066	333.2067	0.30	7.92
PGT-TP2	$C_{21}H_{30}O_4$	346.2144	$[M-H]^-$	$C_{21}H_{29}O_4$	345.2066	345.2073	2.03	33.26
Tramadol (TMD)	$C_{16}H_{25}NO_2$	263.1885	$[M+H]^+$	$C_{16}H_{26}NO_2$	264.1964	264.1959	-1.89	36.84
TMD-TP1	$C_{16}H_{25}NO_3$	279.1834	$[M+H]^+$	$C_{16}H_{26}NO_3$	280.1913	280.1910	-1.07	15.26
TMD-TP2	$C_{16}H_{23}NO_3$	277.1678	$[M+H]^+$	$C_{16}H_{24}NO_3$	278.1756	278.1758	0.72	15.40
TMD-TP3	$C_{15}H_{23}NO_2$	249.1729	$[M+H]^+$	$C_{15}H_{24}NO_2$	250.1807	250.1804	-1.20	19.19
TMD-TP4	$C_{14}H_{18}O_4$	250.1205	$[M-H]^-$	$C_{14}H_{17}O_4$	249.1127	249.1135	3.21	32.31
TMD-TP5	$C_{14}H_{21}NO_2$	235.1572	$[M-H]^-$	$C_{14}H_{20}NO_2$	234.1494	234.1489	-2.14	15.53
TMD-TP6	$C_{14}H_{18}O_3$	234.1256	$[M+H]^+$	$C_{14}H_{19}O_3$	235.1334	235.1335	0.43	21.20
TMD-TP7	$C_{15}H_{21}NO_3$	263.1521						

Table 5.12: LC-Q-TOF-MS data obtained for the detected compounds using molecular-feature extraction database search (continued)

Venlafaxine (VFX)	$C_{17}H_{27}NO_2$	277.2042	$[M+H]^+$	$C_{17}H_{28}NO_2$	278.2120	278.2116	-1.44	24.22
VFX-TP1	$C_{17}H_{27}NO_3$	293.1991	$[M+H]^+$	$C_{17}H_{28}NO_3$	294.2069	294.2064	-1.70	21.64
Bezafibrate (BZR)	$C_{19}H_{20}ClNO_4$	361.1081	$[M+H]^+$	$C_{19}H_{21}ClNO_4$	362.1159	362.1165	1.66	32.64
BZR-TP1	$C_{10}H_{10}ClNO_3$	227.0349	$[M+H]^+$	$C_{10}H_{11}ClNO_3$	228.0427	228.0419	-3.51	11.12
BZR-TP2	$C_{17}H_{18}ClNO_6$	367.0823						
BZR-TP3	$C_{19}H_{20}ClNO_6$	393.0979	$[M-H]^-$	$C_{19}H_{19}ClNO_6$	392.0901	392.0909	2.04	25.91
BZR-TP4	$C_{19}H_{20}ClNO_7$	409.0928	$[M+H]^+$	$C_{19}H_{21}ClNO_7$	410.1007	410.1015	1.95	31.06

Table 5.13: Peak areas obtained for compounds in different water matrices using LC-Q-TOF-MS instrument

Compound	Peak area			
	WW before ozonation	WW after ozonation	WW final effluent	Surface water
Bisphenol A (BPA)	762330	129237	53708	22835
BPA-TP1	ND	30572	ND	ND
BPA-TP2	ND	68654	29718	8492
BPA-TP3	ND	71830	36586	11036
BPA-TP4	ND	ND	ND	ND
BPA-TP5	ND	ND	ND	ND
Caffeine (CAFF)	818050	381696	137185	94725
CAFF-TP1	ND	165986	ND	ND
CAFF-TP2	ND	303756	ND	ND
CAFF-TP3	ND	ND	ND	ND
CAFF-TP4	ND	22623	ND	ND
CAFF-TP5	ND	61360	ND	ND
CAFF-TP6	ND	87568	ND	ND
Estrone sulfate (EST-S)	1583923	763315	352764	290138
EST-S-TP1	ND	738145	351032	105817
EST-S-TP2	ND	258088	160371	75324
EST-S-TP3	12952	574894	217482	146392
EST-S-TP4	ND	472706	216204	136288
EST-S-TP5	ND	79844	ND	ND
EST-S-TP6	ND	206490	ND	ND
EST-S-TP7	ND	847592	194825	141294
EST-S-TP8	ND	304785	ND	95381
EST-S-TP9	71083	403992	152972	ND
Trimethoprim (TMP)	15588353	6890242	2540171	1160638
TMP-TP1	ND	331223	107382	73829
TMP-TP2	ND	ND	ND	ND
TMP-TP3	ND	654672	340173	107384
TMP-TP4	ND	ND	ND	ND
TMP-TP5	ND	1702639	853702	382941
TMP-TP6	ND	ND	ND	ND
TMP-TP7	ND	446738	ND	ND
TMP-TP8	ND	111624	ND	ND
TMP-TP9	ND	ND	ND	ND
TMP-TP10	ND	603150	108204	ND
TMP-TP11	ND	1919237	961730	417392
TMP-TP12	ND	4193945	1503817	ND
TMP-TP13	ND	ND	ND	ND
TMP-TP14	84912	3051127	130729	ND
TMP-TP15	ND	49929	ND	ND
Roxithromycin (ROX)	14733337	2319396	1462941	209049
ROX-TP1	ND	5001038	3171962	1162932
ROX-TP2	ND	564813	ND	ND
ROX-TP3	ND	127267	ND	ND

Table 5.13: Peak areas obtained for compounds in different water matrices using LC-Q-TOF-MS instrument (continued)

ROX-TP4	ND	529349	149827	ND
ROX-TP5	51482	1709411	850824	107273
4-methyl-1H-benzotriazole (4-MBZ)	10235604	3238204	1101159	769635
4-MBZ-TP1	ND	94713	ND	ND
4-MBZ-TP2	ND	48902	ND	ND
4-MBZ-TP3	ND	3957181	974103	811639
4-MBZ-TP4	ND	147926	ND	ND
4-MBZ-TP5	ND	93301	ND	ND
4-MBZ-TP6	ND	ND	ND	ND
4-MBZ-TP7	ND	ND	ND	ND
4-MBZ-TP8	ND	ND	ND	ND
4-MBZ-TP9	ND	ND	ND	ND
4-MBZ-TP10	ND	ND	ND	ND
4-MBZ-TP11	ND	ND	ND	ND
Imazalil (IMZ)	137295	58095	ND	ND
IMZ-TP1	ND	11393	ND	ND
IMZ-TP2	ND	ND	ND	ND
IMZ-TP3	ND	85311	ND	ND
IMZ-TP4	ND	73925	11806	ND
Ketoprofen (KPR)	3784129	228297	134747	74750
KPR-TP1	ND	ND	ND	ND
KPR-TP2	ND	77064	ND	ND
KPR-TP3	61937	1977094	921852	69038
KPR-TP4	ND	108287	ND	ND
Levofloxacin (LVX)	2934988	1049104	610047	482730
LVX-TP1	ND	ND	ND	ND
LVX-TP2	ND	572409	194720	117594
LVX-TP3	ND	253046	ND	ND
LVX-TP4	27510	612551	392581	217047
LVX-TP5	ND	ND	ND	ND
LVX-TP6	ND	96438	38174	ND
LVX-TP7	ND	23706	ND	ND
Chlorophene (CLP)	3219428	193636	ND	ND
CLP-TP1	ND	522592	135039	53852
CLP-TP2	ND	47854	ND	ND
CLP-TP3	ND	ND	ND	ND
CLP-TP4	ND	947140	51482	24928
CLP-TP5	ND	224685	145920	ND
CLP-TP6	ND	405367	ND	ND
CLP-TP7	ND	ND	ND	ND
CLP-TP8	ND	176063	28716	ND
CLP-TP9	ND	675430	191046	ND
CLP-TP10	ND	110585	ND	ND
Acyclovir (ACV)	986501	391101	86105	ND
ACV-TP1	ND	281429	ND	ND

Table 5.13: Peak areas obtained for compounds in different water matrices using LC-Q-TOF-MS instrument (continued)

1*H*-benzotriazole (BZT)	3271285	961734	645675	471384
BZT-TP1	ND	491530	320481	ND
BZT-TP2	ND	ND	ND	ND
BZT-TP3	ND	128894	82613	46910
BZT-TP4	ND	51791	ND	ND
BZT-TP5	ND	ND	ND	ND
BZT-TP6	ND	ND	ND	ND
BZT-TP7	ND	ND	ND	ND
Methylindole (MLD)	1030875	415362	203975	136729
MLD-TP1	ND	19305	ND	ND
MLD-TP2	26849	558115	173972	ND
MLD-TP3	ND	135868	ND	ND
MLD-TP4	ND	122155	ND	ND
MLD-TP5	ND	163077	93021	47285
Imidacloprid (ICR)	349061	139503	116119	14407
ICR-TP1	ND	ND	ND	ND
ICR-TP2	ND	ND	ND	ND
ICR-TP3	ND	84196	ND	ND
ICR-TP4	ND	ND	ND	ND
ICR-TP5	ND	ND	ND	ND
ICR-TP6	ND	60974	ND	ND
ICR-TP7	ND	42143	ND	ND
ICR-TP8	ND	ND	ND	ND
ICR-TP9	ND	ND	ND	ND
Propranolol (PRL)	26223227	14270423	2075446	1289726
PRL-TP1	ND	623672	218319	ND
PRL-TP2	ND	275805	ND	ND
PRL-TP3	ND	1135072	549127	129061
PRL-TP4	ND	907574	459182	171078
Carbamazepine (CBZ)	57081435	20489929	17341322	13190381
CBZ-TP1	ND	789916	ND	ND
CBZ-TP2	78269	4918603	849137	466558
CBZ-TP3	ND	450302	ND	ND
CBZ-TP4	ND	365442	ND	ND
CBZ-TP5	ND	1285809	769638	204676
CBZ-TP6	84104	1440487	483891	ND
CBZ-TP7	ND	682459	160932	ND
CBZ-TP8	ND	854816	529160	150342
CBZ-TP9	ND	75895	ND	ND
CBZ-TP10	ND	127209	ND	ND
CBZ-TP11	ND	239823	ND	ND
CBZ-TP12	ND	ND	ND	ND
CBZ-TP13	ND	12299	ND	ND
CBZ-TP14	ND	806706	440179	172961
CBZ-TP15	ND	32254	ND	ND

Table 5.13: Peak areas obtained for compounds in different water matrices using LC-Q-TOF-MS instrument (continued)

Triclosan (TCS)	9471036	3756209	1603852	971844
TCS-TP1	ND	58401	ND	ND
TCS-TP2	ND	ND	ND	ND
TCS-TP3	ND	819362	ND	ND
TCS-TP4	ND	ND	ND	ND
Aminopyrine (AMP)	6051791	1295179	333184	142216
AMP-TP1	ND	ND	ND	ND
AMP-TP2	ND	ND	ND	ND
AMP-TP3	ND	ND	ND	ND
AMP-TP4	ND	ND	ND	ND
AMP-TP5	ND	ND	ND	ND
AMP-TP6	ND	699876	125648	79550
AMP-TP7	ND	206801	122661	ND
AMP-TP8	ND	720856	252127	19899
AMP-TP9	ND	146573	ND	ND
AMP-TP10	ND	ND	ND	ND
AMP-TP11	ND	576390	ND	ND
AMP-TP12	78126	618046	147872	ND
AMP-TP13	ND	153155	ND	ND
Clarithromycin (CMC)	179370262	85271934	51294611	13839528
CMC-TP1	ND	8181717	772146	133325
CMC-TP2	ND	ND	ND	ND
Atenolol (ATL)	7662520	2985864	1542781	600184
ATL-TP1	ND	35369	ND	ND
ATL-TP2	ND	645487	209614	127403
ATL-TP3	ND	622554	288105	ND
ATL-TP4	ND	304221	ND	ND
ATL-TP5	ND	ND	ND	ND
ATL-TP6	ND	813595	314701	239925
ATL-TP7	ND	ND	ND	ND
ATL-TP8	ND	479514	322915	147972
ATL-TP9	ND	434989	ND	ND
17β-Estradiol (ESD)	37276031	15547844	8975829	1085064
ESD-TP1	51219	1521119	876815	361898
ESD-TP2	ND	735936	347685	ND
Diclofenac (DFC)	77366189	21401404	9305391	6463740
DFC-TP1	ND	5365976	3090425	2283741
DFC-TP2	ND	57607	ND	ND
Metoprolol (MPL)	87524001	40063093	26812037	14736202
MPL-TP1	ND	601043	177572	ND
MPL-TP2	ND	264238	ND	ND
MPL-TP3	ND	518536	288764	ND
MPL-TP4	88434	1677484	756027	204778
MPL-TP5	ND	168770	ND	ND
MPL-TP6	ND	ND	ND	ND

Table 5.13: Peak areas obtained for compounds in different water matrices using LC-Q-TOF-MS instrument (continued)

MPL-TP7	ND	ND	ND	ND
Sulfamethoxazole (SMZ)	14487916	5121821	3421006	2122616
SMZ-TP1	ND	40926	ND	ND
SMZ-TP2	ND	1537973	59209	ND
SMZ-TP3	ND	2204444	706005	320463
SMZ-TP4	ND	ND	ND	ND
Ciprofloxacin (CFX)	1033647	354843	226893	95126
CFX-TP1	ND	9976	ND	ND
CFX-TP2	ND	49387	13628	ND
CFX-TP3	ND	22347	6975	4546
CFX-TP4	ND	19245	6628	3545
CFX-TP5	ND	23157	7420	ND
CFX-TP6	ND	26028	ND	ND
CFX-TP7	ND	10674	ND	ND
CFX-TP8	ND	ND	ND	ND
CFX-TP9	ND	65310	ND	ND
CFX-TP10	ND	ND	ND	ND
CFX-TP11	ND	ND	ND	ND
CFX-TP12	ND	ND	ND	ND
CFX-TP13	ND	ND	ND	ND
CFX-TP14	ND	22719	14410	ND
CFX-TP15	4074	54861	24076	17222
CFX-TP16	ND	22274	ND	ND
Norfloxacin (NFX)	8354410	2121008	980437	848464
NFX-TP1	ND	435276	ND	ND
NFX-TP2	ND	39751	ND	ND
NFX-TP3	ND	786872	299916	128136
NFX-TP4	ND	716277	440794	137372
NFX-TP5	ND	ND	ND	ND
NFX-TP6	ND	ND	ND	ND
NFX-TP7	ND	529395	187421	ND
NFX-TP8	ND	ND	ND	ND
NFX-TP9	ND	ND	ND	ND
NFX-TP10	ND	170884	104533	38293
NFX-TP11	ND	80479	ND	ND
NFX-TP12	ND	121486	74218	ND
NFX-TP13	ND	510187	98293	66737
NFX-TP14	ND	32093	ND	ND
NFX-TP15	ND	66539	ND	ND
Paracetamol (PCM)	62169628	30491863	17126342	6506015
PCM-TP1	ND	8172924	2826511	936328
Acesulfame (ACF)	9743236	3563214	860603	261659
ACF-TP1	ND	505524	ND	ND
Cephalexin (CPX)	3196208	809421	327194	87079
CPX-TP1	ND	93184	40790	ND

Table 5.13: Peak areas obtained for compounds in different water matrices using LC-Q-TOF-MS instrument (continued)

CPX-TP2	ND	75107	ND	ND
CPX-TP3	ND	32387	ND	ND
Penicillin G (PG)	5012305	2165469	807900	167935
PG-TP1	ND	1891004	1010924	ND
Progesterone (PGT)	6167935	1266133	408936	167912
PGT-TP1	ND	1354894	889716	53848
PGT-TP2	ND	1079206	ND	ND
Tramadol (TMD)	54061728	6611543	5885412	3990035
TMD-TP1	ND	7177516	2546070	980532
TMD-TP2	ND	1000557	597208	306875
TMD-TP3	30717	2094721	691728	ND
TMD-TP4	ND	1650152	ND	ND
TMD-TP5	ND	2353811	1183445	632704
TMD-TP6	ND	176445	ND	ND
TMD-TP7	ND	ND	ND	ND
Venlafaxine (VFX)	34824989	14214829	3509104	2812246
VFX-TP1	ND	439091	194612	70409
Bezafibrate (BZR)	11427071	4733424	2140821	1190497
BZR-TP1	ND	226576	111439	79942
BZR-TP2	ND	ND	ND	ND
BZR-TP3	ND	851374	120158	ND
BZR-TP4	ND	27659	ND	ND

5.3.1 References

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5.4 General conclusions and outlook

No supplements

5.5 List of publications

Publications in peer-reviewed journals

A. A. Deeb, T. C. Schmidt:
Tandem anion and cation exchange solid phase extraction for the enrichment of micropollutants and transformation products from ozonation
Analytical and Bioanalytical Chemistry, (2015), Submitted.

Other Publications

A. Deeb, M. K. Fayyad, M. A. Alawi:
Separation of Polyphenols from Jordanian Olive Oil Mill Wastewater
Chromatography Research International, (2012), 2012.

Poster presentations

A. A. Deeb, T. C. Schmidt:
Solid phase extraction strategies for the enrichment and isolation of micropollutants and their transformation products from different water matrices
Istanbul (Turkey), 5th EuCheMS Chemistry Congress, 31 August – 4 September, 2014
TOP POSTER AWARD was given.

A. A. Deeb, T. C. Schmidt:
Solid phase extraction strategies for the enrichment and isolation of micropollutants and their transformation products from different water matrices
Barcelona (Spain), SETAC Europe 25th Annual Meeting, 3 – 7 May, 2015.

5.6 Curriculum Vitae

Der Lebenslauf ist in der Online-Version aus Gründen des Datenschutzes nicht enthalten.

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5.8 Erklärung

Hiermit versichere ich, dass ich die vorliegende Arbeit mit dem Titel:

„Target and suspect screening of organic micropollutants and their transformation products in aqueous samples"

selbst verfasst und keine außer den angegebenen Hilfsmitteln und Quellen benutzt habe, und dass die Arbeit in dieser oder ähnlicher Form noch bei keiner anderen Universität eingereicht wurde.

Essen, im Februar 2016

Ahmad Deeb